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CONTENTS

PAGE

EXPERIMENTS WITH MAMMALIAN LEUKEMIA. *Maurice N. Richter and E. C. MacDowell*.. 509

RECENT PROGRESS IN INSECT PHYSIOLOGY. *W. M. Hoskins and R. Craig*..... 525

VARIATIONS IN WHITE BLOOD CELL COUNTS. *W. F. Garrey and W. Ray Brown*..... 597

THE SO-CALLED ORGANIZER AND THE PROBLEM OF ORGANIZATION IN AMPHIBIAN DEVELOPMENT. *Paul Weiss*..... 639

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PHYSIOLOGICAL REVIEWS

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EXPERIMENTS WITH MAMMALIAN LEUKEMIA[1]

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It is nearly 90 years since the first case of leukemia in man was described. In the interval, progress has been made in the clinical recognition of cases, the separation of subvarieties, and the descriptive pathology of the disease. But the nature of the condition—whether infectious or neoplastic—is still under dispute. On this point some of the more recent experimental work has a direct bearing.

The essential histopathologic changes in leukemia may be described as an overproduction of white blood corpuscles, the failure of these cells to mature, their entrance into the blood stream in greatly increased numbers, and their appearance in the interstitial tissues of organs in which blood formation is not normally found in the adult. With the recognition of several varieties of white cells, corresponding varieties of leukemia have been described, according to the cell type involved.

Studies on animal leukemias advanced beyond the descriptive phase in 1908, when Ellermann and Bang reported the transmission of fowl leukemia by a filtrable agent. In mammals, however, experiments yielded little information of value until within the last decade, when observations on the occurrence and transmission of leukemia have been reported from several widely separated laboratories.

Terminology. The recent literature from the experimental field indicates that the conditions of lymphoid hyperplasia (neoplasia?) designated by the terms leukemia, pseudoleukemia, lymphosarcoma, leukosarcomatosis, or the less common but probably more accurate terms lymphadenosis (leukemic or aleukemic) or lymphomatosis, are varieties of the

[1] The experimental work of the reviewers and their associates, included herein, is part of a coöperative project supported by a fund from the Carnegie Corporation and appropriations from the Research Fund of Columbia University.

same fundamental condition, differing in the distribution of lesions. Unless otherwise indicated, the term leukemia will be used in reference to all of these conditions, including the myeloid forms, but not to Hodgkin's disease.

SPONTANEOUS LEUKEMIA. For reviews of the occurrence of leukemia in animals see Opie (1928), Knuth (1929), and Jarmai (1934).

A. Constitutional influence. There have been several suggestions that leukemia in man is influenced by constitutional factors, as indicated by the occasional occurrence of more than one case in a family. Petri (1931), however, found only 11 undoubted instances in the literature. The data are insufficient for drawing conclusions.

In animals, the only suggestions of constitutional factors have been in lymphatic leukemias occurring in certain herds of cattle in East Prussia (reviewed by Knuth, 1929) and in mice. In cattle, genetic studies have not been made. In mice, the constitutional factors have been investigated by Slye (1931) and MacDowell and Richter (1935).

a. Genetic experiments. Slye observed cases of mouse leukemia only in "tumor strains." She regards leukemia as a neoplasm, and believes that the genetic basis of all neoplasia is a recessive Mendelian gene.

Three objections may be raised to Slye's conclusions.

1. The paper on leukemia presents only a portion of the cases observed (44 of 975). Complete data are necessary in this instance, for the conclusions are based on the ex post facto interpretation of pedigrees, rather than on experimental tests.

2. The hypothesis has not been tested by crosses between genetically homogeneous strains, as required for a genetic conclusion.

3. Genetic ratios are supposed to be indicated by the somatic condition of the mice—i.e., leukemic or non-leukemic. In view of the results obtained by MacDowell and Richter, mentioned below, the occurrence of leukemia does not necessarily indicate the genetic condition of the animal. For the determination of genetic ratios, breeding tests of individuals in a segregating generation are necessary.

The only pedigreed "leukemic" strain with data available for genetic analysis is that developed by MacDowell and studied by MacDowell and Richter (1935). In this strain (designated as C58) inbreeding by brother-sister matings had been carried out for 18 generations before the start of investigation on leukemia, resulting in a high degree of genetic uniformity. In the 18th to 23rd generations about 90 per cent (omitting doubtful cases) of all mice surviving more than 6 months developed leukemia spontaneously.

In a group of 543 leukemic cases, 450 were lymphatic in type, 6 were myeloid, and in 87 the type could not be determined, in most instances because of postmortem degeneration.

In certain other strains of mice bred in the same manner and in the same laboratory, leukemia has appeared, but only sporadically. One strain (Storrs-Little) has had only 4 cases (1.3 per cent).

In strain C58, the negative and doubtful cases are scattered at random in the pedigree, and the incidence of leukemia in their offspring is as great as in the offspring of leukemic mice.

In hybrids of the F_1 generation of a cross between males of strain C58 and females of strain Storrs-Little, the incidence of leukemia was about half that of the pure strain C58 (42 per cent of 106 mice, omitting doubtfuls), and in the back cross generation (F_1 males mated to Storrs-Little females) about $\frac{1}{4}$ of that in C58 (20 per cent of 96 mice). Thus with leukemia transmitted through the fathers, the incidence in hybrids is roughly parallel to the total heredity from strain C58. The occurrence of leukemia in F_1 hybrids indicates that the genetic difference between these strains is not a single recessive Mendelian gene.

b. Non-chromosomal influences. The random scattering of leukemias in the pedigree of strain C58, and the similar incidence of the disease in the offspring of leukemic and non-leukemic mice, indicate genetic uniformity. Therefore, the failure of 10 per cent of the mice to develop leukemia is due to non-genetic factors. Similarly, in an F_1 generation, the animals are genetically alike. Hence, in the F_1 generation of the cross between C58 and Storrs-Little mice, the failure of about half the mice to develop leukemia is due to non-genetic influences. It may be said that in C58 mice genetic factors are about 9 times as strong as non-genetic factors in determining the occurrence of leukemia, while in the F_1 hybrids of the cross mentioned, genetic and non-genetic factors are equally strong.

One non-genetic variable capable of influencing the incidence of leukemia in the offspring is transmitted through the mother, for the incidence in the F_1 generation is 19.4 per cent higher when C58 females instead of males are mated to Storrs-Little mice (61.9 per cent vs. 42.5 per cent). A similar phenomenon is observed in the back-cross generation (F_1 mated to Storrs-Little), for the incidence is 26.7 per cent higher when the hybrid parent is female than when it is the male (46.5 per cent vs. 19.8 per cent).

B. External influences. a. Bacteria. Young (1922) isolated a pleomorphic organism from leukemic tissues of mouse and man and from

human cancers. Inoculation of mice with cultures of this organism was followed by the development of a number of cases of leukemia. The mice used were obtained from two dealers, were of all colors and ages, but chiefly over 6 months. With the organism obtained from mice 179 mice were inoculated, and with the human organism 40 mice, but the conclusions are based on 96 and 16 mice respectively, the discrepancy partly due to deaths with "toxic lesions" a short time after inoculation and partly to mice still alive. Of the 96 inoculated with the mouse organism, 74 died of leukemia; of the 16 inoculated with the human organisms 12 died of leukemia and 2 of cancer. Although it is stated that the controls were equal in number to the inoculated animals, the only results reported are for 11 mice of which 2 had leukemia. The results are given in such a way that it is impossible to make a direct comparison between the controls and the inoculated animals. Under these circumstances the influence of the injections cannot be determined.

Cherry (1929) inoculated mice with tubercle bacilli by an intradermal technique. The inoculated mice and the controls were each divided into two groups according to whether the milk with which they were fed was raw or boiled. The incidence of leukemia in the four groups is given as follows: raw milk inoculated, 39.2 per cent (of 51 mice); raw milk controls, 37.2 per cent (of 51 mice); boiled milk inoculated, 20.0 per cent (of 45 mice); boiled milk controls, 11.0 per cent (of 100 mice). Tumors, other than leukemia, also appear more frequently in the inoculated than in the corresponding control groups, and more frequently in the raw milk than the boiled milk groups.

Cherry believes that infection with tubercle bacilli was an important factor in the incidence of neoplasms, and of leukemia in particular. Several facts, however, largely invalidate this claim: although it is stated that the incidence of leukemia in the mouse stock was low, the mice used were descendents of those reported by Hill (1930) in which about 60 per cent developed leukemia spontaneously; an unknown amount of foreign blood was brought into the stock; the mice were not pedigreed and genetic analysis of the results is impossible; the control mice (boiled milk) were not adequate, for they were of younger average age, and apparently did not live concurrently with those inoculated. It is thus impossible to determine the relative importance of hereditary and non-hereditary factors in the cases observed.

In a subsequent paper, Cherry (1931) reported that in 349 inoculated mice leukemia occurred 34 times, and in 305 control mice only 3 times. The report does not indicate whether or not these figures include those of the earlier paper.

b. Chemicals. Lignac (1928) inoculated white mice with benzol in olive oil, in repeated small doses. In one series of 6 animals inoculated from 5 to 33 times, lymphoid hyperplasia of the spleen was observed, but the mice did not have leukemia. Continued administration of benzol caused lymphoid atrophy. In 3 mice receiving 50 injections, multicentric neoplastic growths of myelopotent stem cells were found in the liver, without changes elsewhere that resembled leukemia.

In a subsequent experiment Lignac (1932) inoculated 54 adult mice with 0.001 cc. benzol in oil. Of these, 8 mice developed leukemia from 4 to 11 months after the onset of the experiment. One mouse had lymphoblastic leukemia, 2 had aleukemic lymphatic leukemia, 2 had mast cell leukemias (one leukemic and one aleukemic), 2 aleukemic myelosis and one eosinophilic leukemia. It is of interest that tissue from one of the cases of aleukemic myelosis was inoculated into 8 mice, one of which developed lymphatic leukemia 4 months later. A further experiment was performed, in which 36 mice were inoculated with a mixture of tar and benzol. One of these died with lymphatic leukemia 8 months after the beginning of the experiment. In a series of 20 mice inoculated with smaller doses of the tar-benzol mixture and subjected to ultraviolet radiation, one developed myeloid leukemia. Of 30 mice inoculated with splenic pulp from this case, one developed myeloid leukemia after 7 months. No information is given about the source of the mice or their genetic relationships, but the statement is made that of 1,465 mice autopsied during the past 9 years, none had leukemia.

Mercier (1930) observed leukemia in mice after injections of tar. The mice used were descended from a single pair. The disease also occurred in uninoculated mice, and data sufficient for the determination of the influence of tar are lacking.

Büngeler (1932) observed that repeated injections of indol in mice resulted in hemolytic anemias with leucopenia. With small doses continued over a long time, reparative phenomena were observed. Of 97 animals surviving 8 months, 17 (17.5 per cent) had leukemia in some form, 4 being lymphatic (3 leukemic, one lymphosarcoma), and 13 myeloid (4 leukemic, 9 aleukemic). In a large number of control mice, only 2 cases of leukemia (lymphatic) were observed. Metabolic studies of non-lymphoid tissues showed that indol caused a decrease in oxygen consumption and an increase in anaerobic glycolysis, such as has been observed in neoplasms.

As mentioned above, the influence of external factors on the incidence of leukemia in mice may vary with the genetic constitution of the

animals. In the opinion of the reviewers, the influence of genetic variables has not been satisfactorily eliminated in the several experiments quoted above, so that the influence of the external agents remains uncertain.

c. Irradiation. Krebs, Rask-Nielsen and Wagner (1930) found that among 10,500 normal mice there occurred 7 cases of leukemia, while among 5,550 subjected to x-rays, there were 19 cases. The source of the mice and methods of breeding are not stated.

Furth (1934) found that myeloid leukemia was rarely found in his mice until many had been exposed to a single dose of 400 r-units of x-rays or smaller doses at monthly intervals. Of 385 irradiated mice, 21 died with myeloid leukemia; of 481 controls, 2 died of myeloid leukemia.

EXPERIMENTAL TRANSMISSION. A number of independent investigations have shown that leukemia may, under certain circumstances, be transmitted from animal to animal. Transmission has been successful in the mouse (Richter and MacDowell, 1929; 1930a; Meyer, 1928; Korteweg, 1929; Furth and Strumia, 1931; Krebs, Rask-Nielsen and Wagner, 1930), the guinea pig (Snijders, 1926; and Tio Tjwan Gie, 1927), and the South American red squirrel (Fraser, 1925).

A. The agent. a. Nature of the agent. A non-cellular agent capable of transmitting mammalian leukemia has not been discovered. No bacterial organism or parasite has been found. In all successful transmissions, leukemic tissues or cells emulsified in physiological solutions have been injected. The only exception to this statement is a single experiment reported by Meyer (1928), who passed a suspension of tissue from mouse leukemia through filter paper, and obtained one transmission with the filtrate among 10 mice inoculated. This result does not warrant Meyer's conclusion that the agent is a filtrable virus.

On the contrary, the filtration experiments of Snijders (1926) and Tio Tjwan Gie (1927) with guinea-pig leukemia, Richter and MacDowell (1933), and Furth, Seibold and Rathbone (1933) with mouse leukemia, indicate that these were not filtrable. The same authors found that the agent would not survive in glycerin, nor would it resist freezing and thawing or mechanical grinding. All of these observations indicate a close relationship between the agent and the leukemic cells. This relationship is further emphasized by the inverse relationship between the number of cells introduced and the interval before death of susceptible animals (Richter and MacDowell, 1933; Furth, Seibold and Rathbone, 1933), and the direct relationship between the number of cells and the percentage of successful inoculations (Furth, Seibold and Rathbone, 1933; MacDowell, Taylor and Potter, 1934).

Krebs, Rask-Nielsen and Wagner (1930) reason by the method of exclusion that the leukemias developing after inoculation result from the proliferation of lymphocytes of the host, but present no direct evidence. With this exception and that of Meyer, mentioned above, all observers have considered the cells of leukemic infiltrations to be descendants of those introduced. Direct cytologic evidence of this fact was obtained by Potter and Richter (1933) who observed the dissemination of the cells from the site of entry by ameboid activity and their entrance into the lymphatic channels.

b. Specificity of the agent. Richter and MacDowell (1930b) and Furth, Seibold and Rathbone (1933) found that with the establishment of several lines[2] of leukemia transmitted by inoculation, certain features appeared in particular lines with such frequency that they could be considered as characteristic of the lines. In the experiments of Richter and MacDowell these features occurred after inoculation of genetically uniform mice, and were due to differences, not in the mice, but in the cells introduced. The features observed may be discussed under several heads, as follows:

1. Specificity in distribution of lesions. In each line of leukemia, there are certain situations in which infiltrations are characteristically found. Thus in two lines studied by Richter and MacDowell (1930b) (lines H and I), the liver was markedly infiltrated in all animals (85 and 98 mice respectively), while in lines A and E some mice (36 and 30 mice) had marked liver involvement, others (64 and 32 mice) had slight infiltration, and still others had none (71 and 11 mice). With regard to infiltration of the kidney, the peripheral blood count, the presence or absence of ascitic or pleural effusions, and the occurrence of local tumors, characteristic differences were also encountered. Potter and Richter (1933) showed that there was, in each line, a sequence in the order of organ involvement, and a characteristic time of infiltration of each organ after inoculation in the same site. Differences in the distribution of lesions in 5 transmission lines were reported by Furth, Seibold and Rathbone (1933), who also found that the distribution is influenced by the site of inoculation.

[2] The term "line" is used to indicate cell lineage in successive transfers. For the sake of uniformity, other terms (e.g., "strain," etc.) used for the same purpose are changed to "line" in this review. The term "strain" is used to indicate the ancestry of animals of the same genetic constitution controlled by pedigreed inbreeding, and "stock" when pen-breeding or other uncontrolled methods are used.

2. Specificity in cell type. In the three lines studied cytologically by Potter and Richter (1933), the leukemic cells varied morphologically, but the types encountered were similar to some stages in the normal development of the lymphocyte. The cells underwent division, but only slight differentiation. The chromosome content was typically 40, which is normal for the mouse, but precocious splitting of the chromosomes was observed; the type of Golgi apparatus was the same in all transmission lines. In each line the type of cell remained constant for numerous transfers with regard to size, shape, average frequency of division, number of mitochondria and nuclear-plasmasome ratio.

Furth, Seibold and Rathbone (1933) observed that in 5 lines, the lymphocytes in the blood had characters distinctive of the line, such as characteristic frequency of vacuolation of nucleus or cytoplasm, azure granules, fragmented nuclei, smudged cells, medium and large cells, and cytoplasmic basophilia. In one line (Ar 21) the features of the predominant cell were so characteristic that a single immature lymphocyte was sufficient to identify the line.

3. Specificity in metabolism. Victor and Potter (1933) studied the metabolism of normal mice of strain C58 and those carrying leukemic cells of lines A and I by Warburg's methods, using Fenn respirometers. The genetic uniformity of the mice used led to greater consistency in repeated observations than is reported by others for neoplastic tissue. It was found that not only are there striking differences between the metabolism of lymph nodes of normal and leukemic animals, but also between those of the two different lines of transmissible leukemia studied. The oxygen consumption of nodes of line A (9 observations) was greater than that of nodes of both line I (10 observations) and normal mice (10 observations). However, the aerobic glycolysis of line I was almost three times as great as that of normal and line A. The anaerobic glycolysis of lymph nodes of line I was about 1.5 times as great as that of line A and almost three times as great as that of normal mice. The differences observed were from 5.8 to 18 times the probable errors of the differences. These observations are of special interest because they are the first to compare the metabolism of neoplastic cells with normal cells of the same kind.

In a subsequent investigation (Victor and Potter, 1934), it was shown that the metabolism of lymph nodes of normal mice of strains C58 and Storrs-Little was essentially alike at the age used for inoculation (6–8 weeks). When, however, leukemic cells of line I were transmitted to mice of strain Storrs-Little instead of the usual C58 hosts, the rate of

oxygen consumption increased, but that of aerobic and anaerobic glycolysis decreased. This difference was not due to changes in the cells, for the same transfer was used for inoculation into hosts of each strain. Another line of cells (line M-liver), originating in strain C58, is similar in its metabolism to line I when carried in C58 mice, but when inoculated into Storrs-Little mice, the rate of oxygen consumption did not change significantly, although aerobic and anaerobic glycolysis were reduced. Thus there are demonstrated differences in the metabolic response of two different lines of cells to the same change in environment. The metabolic changes produced by different host constitution are reversible, for the rates returned to significantly the original level when, after several transfers in Storrs-Little, the cells were again inoculated into hosts of strain C58. Thus the metabolic rate observed for a given line of cells is not a trait of the line as such, but rather the reaction of a line of cells to a certain host.

Cell metabolism in spontaneous cases is more variable than in any of the lines studied (Victor and Potter, 1935a). Leukemic tissues both oxidize and ferment glucose, as evidenced by increase in respiratory quotients and glycolysis in the presence of glucose. The oxygen consumption and glycolytic rates of leukemic tissues from spontaneous cases are higher than in lymphoid tissue of normal mice at comparable ages. Victor and Potter (1935b) found that the rate of glycolysis was greater in older (6–9 months) normal mice of strain C58 than in younger (6–8 weeks) normal mice of the same strain. This change with age was not observed in four other inbred strains with comparatively low incidence of leukemia, and suggests that the metabolic changes precede the malignant ones.

4. Specificity in host requirements. Leukemic cells do not grow in all mice into which they are inoculated. The influence of the genetic constitution of the host on the survival and growth of inoculated cells is discussed under the heading of "The host. Factors influencing susceptibility."

c. Changes in characteristics. When the transfers are made in genetically uniform hosts, there is a tendency for uniformity in results, particularly after the first few transfers. Nevertheless changes may occur at any time, and may involve any of the features that hitherto had been characteristic. Thus, in several lines arising in strain C58 (Richter and MacDowell, 1930b) there were changes in the distribution of lesions, in the occurrence of such phenomena as pleural or peritoneal effusions, the presence and degree of elevation in leucocyte counts, the occurrence

of local tumor-like growths, the predominant cell type, the interval between inoculation and death, the degree of variability, cell metabolism, and host requirements. The genetic and ontogenetic uniformity of the mice used in their experiments indicate that the changes observed occurred in the cell lines, and not in the mouse strains. This point is controlled by other lines carried in mice of the same strain at the same time.

In all reported transmission lines, the interval between inoculation and death has decreased during the first few transfers, a phenomenon generally referred to as an increase in virulence. In genetically uniform mice, this interval subsequently becomes a characteristic feature of the transmission line (Richter and MacDowell, 1930b; Potter and Richter, 1933), and may remain remarkably uniform for many transfers. These observers found that the interval between inoculation and death of uniform mice gives more accurate information of changes in virulence than does the proportion of susceptible animals in a segregating generation.

The decrease in interval occurring during the first few transfers may be followed by a long period of uniform intervals, or there may be further decreases at any time, or very rarely, increases. Richter and MacDowell (1934) measured the cells in the infiltrations during periods of abrupt changes in interval, dividing them into 4 classes, and found that the proportions of the different classes changed gradually and preceded the interval change. When the proportion of large cells increased, a decrease in the interval followed, and vice-versa. The interval changes were apparently dependent on the changes in cell population.

Changes in host requirements have led to difficulties in the interpretation of genetic experiments. Thus, whereas the requirements for survival of cells of one line (line I) in hybrids (back-cross generation) of strains C58 and Storrs-Little remained constant during several experiments, the host requirements for another line of cells (line A) varied when tested at different times (MacDowell and Richter, 1930). Line I cells would not at first grow in Storrs-Little mice, but after being carried for 103 transfers (28 months) in C58 mice, they grew in about half of the inoculated Storrs-Little animals. As an analysis of host requirements can be made only during periods of stability of the cells, and as the latter condition can not be determined accurately, a complete analysis of the conditions appears to be impossible.

Furth, Seibold and Rathbone also observed changes during the course of transfers, most occurring during the first few passages. For

example, they found changes in the incubation period and duration of illness, the characters of the cells, and the percentage of successful inoculations. They suggested that selection of the more rapidly growing or more resistant cellular offspring occurs during transfers, and may be the main factor responsible for the changes that occur during the first passages. In this connection, it is of interest that Victor and Wintersteiner (1934) found metabolic differences between lines originating in the same spontaneous case and carried by parallel transfers in genetically uniform mice. But whether these results are due to selection from a mixed population of cells or to changes occurring within a subline remains an open question.

B. The host. a. Factors influencing susceptibility. 1. Species. Without exception, the transmission of leukemia to normal animals has been successful only within the same species. This species specificity has so far been overcome only by irradiation of the animals before inoculation, as in the transmission of mouse leukemia to the rat by Krebs and Thrane (1932) and Furth, Seibold and Rathbone (1933).

2. Strain. Even within the same species transmissibility may vary from 0 to 100 per cent. The highest figures were obtained by Richter and MacDowell in the particular inbred strain of mice known as C58, mentioned above. Practically all individuals of this strain are susceptible to inoculation of leukemic cells originating in spontaneous cases in the strain. In another strain of mice (strain Storrs-Little), the lines of leukemic cells arising in and carried by mice of strain C58 could not be grown except as noted above.

A genetic analysis of the factors differentiating resistance and susceptibility was attempted by mating C58 and Storrs-Little animals and determining the susceptibility of their offspring.

In testing for susceptibility to the cells of line I, it was found (MacDowell and Richter, 1932) that all mice of strain C58 were susceptible (1239 tested), but none of Storrs-Little (273 tested). The F_1 hybrids were all (177 tested) susceptible. In the back-cross generation (F_1 mated with Storrs-Little) slightly less than half were susceptible (362–389). The excess of survivors above 50 per cent is not a statistically significant deviation from a 1:1 ratio, and the results indicate the differential action of a single dominant gene for susceptibility. Nevertheless, the excess of survivors was consistent in all experiments, and indicates that a few heterozygous mice in the back-cross generation may survive inoculation, whereas those in the F_1 generation do not. The second back-cross and F_2 generations were studied to determine the cause

of the excess survivors in the back-cross. If a single gene is involved, half of the back-cross mice should transmit susceptibility to half their offspring, the other half should not transmit susceptibility to any of their offspring. In 50 back-cross survivors and 1326 of their offspring, it was found that 25 back-cross gave offspring that were susceptible in per cents ranging from 30 to 60, while of the other 25, 19 gave no susceptible young, and the other 6 gave only 1 to 6 per cent of them. After breeding, the 50 back-cross parents were inoculated. Seventeen were susceptible and all of these had given susceptible offspring in the ratio of about 1:1. In the F_2 generation, there were 62 susceptible and 16 resistant mice, the deviation from 3:1 ratio (the expected ratio for a single gene) being not statistically significant. The authors conclude that the primary differential between strains C58 and Storrs-Little in respect to susceptibility to the leukemia of line I is a single dominant, autosomal gene. The excess of survivors in the back-cross and second back-cross generations above the expected ratios is explained by the fact that the two strains also differ in other genes, the segregation of which occasionally permits a mouse carrying the primary gene for susceptibility to survive. The segregation of other genes also explains the occasional occurrence of a mouse transmitting susceptibility to only a very small proportion of its offspring.

In analyzing susceptibility to cells of line A, the results differed in three particulars from those of line I: there were some mice that became ill but recovered after inoculation; the proportion of susceptible back-cross mice was considerably less; this proportion changed in different experiments. In 18 experiments involving 528 mice, the ratios of susceptible to resistant mice in the back-cross generation varied from 1:2.5 to 1:124, approximating the ratios for from 2 to 7 independent genes. The changes in ratio, however, make it impossible to verify results.

Additional evidence of the influence of heredity on susceptibility may be shown by comparison of other lines of cells in different strains. Thus MacDowell and Richter (1931) obtained a line of cells (line L) originating in a spontaneous case in an unrelated strain (strain 89) of mice. The inoculation of 75 mice of strain 89 in 13 transfers of line L cells gave 71 (94.6 per cent) susceptible, but none of 64 inoculated mice of strain Storrs-Little, and only one of 82 mice of strain C58 were susceptible to the same line of cells. On the other hand, cells of line I (originating in strain C58) grew in all C58 mice, none of Storrs-Little (until the 103rd transfer, as mentioned above) and in a large proportion (94 of

142) of strain 89 mice. Thus a particular mouse may be susceptible to a particular line of leukemia, but not to another line. Furth, Seibold and Rathbone (1933) found that of 5 transmission lines from 3 stocks of mice, 3 could be transmitted only to mice closely related to those in which the lines arose (mice of the same inbred, but not pedigreed stock), and 2 (from spontaneous cases in stock A) could be transmitted to mice of all 3 stocks. Their line A35 (arising in stock A) grew in 127 of 352 normal stock A mice inoculated intravenously, and 5 of 29 stock A mice inoculated subcutaneously. In stocks R and S intravenous inoculation was successful in 6 of 21 mice and in 3 of 34 mice, respectively. The percentage of successful transfers varied at different times even when using mice of the same stock and cells of the same line, and varied as much as from 0 to 100 per cent in different families of the same stock.

3. Irradiation. Krebs, Rask-Nielsen and Wagner (1930) found that lymphatic leukemia in mice could be transmitted to their non-leukemic mice only if the animals were previously irradiated. In this way they succeeded in carrying one line of cells for 25 transfers, and other lines for shorter periods.

Furth, Seibold and Rathbone (1933) found that most, if not all resistant mice can be made susceptible through exposures to quantities of x-rays (400 to 600 r) just below the lethal dose. None of 168 non-irradiated mice of their stock A developed leukemia after inoculation of cells of line Rg10, but transmission was successful in 27 of 30 irradiated mice. The percentage of mice rendered susceptible by irradiation is less when the quantity of irradiation is decreased, but with the technique used, 30 r-units or about $\frac{1}{20}$ lethal dose was sufficient to make a few mice susceptible. The interval between inoculation and death was shorter in mice receiving larger doses of x-rays.

The duration of susceptibility of the mice inoculated with line Rg10 cells increased with the number of r-units, and in the case of large doses (1200 units) susceptibility lasted (in 1 of 2 mice) for 103 days.

Irradiation 2 hours after inoculation did not render mice of stock A susceptible to line Rg10, but slightly increased the proportion of susceptible mice in stock R. Of 13 non-irradiated mice of stock R, 2 developed leukemia after inoculation. When irradiated 2 hours after inoculation, 5 of 13 mice developed leukemia. The interval before death was increased from 11 days (average of 4 non-irradiated mice) to 15.8 days (average of 11 irradiated mice) by irradiation after inoculation. With cells of line A35, similar results were obtained, the main result being increased interval between inoculation and death in the irradiated mice.

The same authors found that rats, which are normally immune to mouse leukemia, could be rendered susceptible by irradiation before inoculation. Transmission was successful in 3 of 6 experiments with line Rg10 cells (6 of 30 irradiated rats). Using line A35, the inoculation of 12 irradiated rats in 3 experiments was unsuccessful. Similarly, blood of a chicken with transmissible leukosis was injected into 5 irradiated and 4 non-irradiated mice, none of which developed leukemia. The transmission of myeloid leukemia and Hodgkin's disease of man to irradiated mice was also unsuccessful, but Furth (1934) transmitted myeloid leukemia of the mouse to irradiated mice with success in 42 of 46 inoculated animals in the first 4 transfers.

4. Immunity. MacDowell, Taylor and Potter (1934) immunized susceptible mice by inoculation with sublethal doses of leukemic cells. During a period of $5\frac{1}{2}$ years, line I cells have been passed into 3625 mice of strain C58, and all but one died of leukemia characteristic of that line. During the last $3\frac{3}{4}$ years, all of 2925 mice inoculated with line I cells developed leukemia. These inoculations were all made with a massive dose, standardized at about 80 million cells. By using dilutions of the standard dose of the order of 4^{-5} down to 4^{-11}, increasing proportions of these mice survived. They were subsequently resistant to progressively larger doses. After 1 to 5 such doses, the standard dose was given without the development of leukemia in 114 out of 118 mice.[1]

THE NATURE OF LEUKEMIA. The principal theories of the nature of leukemia concern its relationship to infection on the one hand, and to neoplasms on the other.

From the experimental side, no conclusive evidence of the influence of any organism has been presented. The results of experiments purporting to show such evidence have already been discussed and interpreted in a different manner. On the other hand, most of the information about mammalian leukemia strongly favors the conception of the disease as a neoplasm. As there is an obvious lack of exact definitions, both of neoplasia and of leukemia, the case rests at present on the similarities between the two diseases.

[1] Recently it has been shown (Rhoades and Miller, *Proc. Soc. Exp. Med.* Mar., 1935) that resistance can be induced by normal tissue, and that (MacDowell, Taylor and Potter, *Proc. Nat. Acad. Sci.*, Aug., 1935) an important factor in this resistance is the genetic constitution of the normal tissue in relation to that of the treated mouse; one combination of genetic constitutions induces resistance, another does not and still another (unpublished) hastens the development of the subsequently inoculated leukemic cells.

There are several obvious parallels between leukemia as studied experimentally in the reports reviewed above, and the experimental study of neoplasms in mammals. From the standpoint of spontaneous leukemia, the feature of greatest prominence is the influence of heredity on the incidence of leukemia and of other neoplasms. Thus the incidence of carcinoma in mice has been shown to vary greatly in different strains, and to be under genetic control, although the mechanism of this control and the part played by non-genetic factors are not clearly established. In crosses between strains of high and low incidence, both carcinoma (Little, 1933) and leukemia show a significantly higher incidence when inherited through the mother than through the father. These two cases of maternal (or non-chromosomal) transmission are the only ones known in mammals.

The parallels are more striking when the transmission experiments are considered, for most of the phenomena observed during the continued passages of leukemic cells in mice have also been observed during the passage of mammalian tumors. For example, both leukemia and tumors are transmissible only by means of living cells; among normal animals, they can be transmitted only to those of the same species, and of a constitution that permits the survival and growth of their cells; this cell-host relationship is specific and different for different lines of cells, and expresses itself not only in terms of susceptibility and resistance, but also in altered morphology or growth characteristics; in both instances increased virulence is manifested after repeated transfers; in both instances cell metabolism is altered; and in both cases early splitting of the chromosomes is observed during mitosis.

The changes in leukemic cells that have occurred during passages as manifested by altered morphology, virulence, host requirements, etc., are also similar to changes that have occurred in the transmission of neoplasms.

The main differences between leukemia and neoplasms, as observed experimentally, are related to the greater independence and motility of the leukemic cells, because of which special vascularization or "stroma reaction" is not required for their growth after inoculation.

Fowl leukemia, which is not reviewed in this report, is transmissible by a filtrable agent resembling in this respect the filtrable tumors in this animal.

Considered from the experimental aspect, there is so great a resemblance between the data obtained from studies of leukemia and neoplasms in animals that little doubt exists in the minds of contemporary workers in this field that the processes underlying the various neoplasias and leukemia differ only in so far as they involve different tissues.

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RECENT PROGRESS IN INSECT PHYSIOLOGY

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The physiology of insects has attracted the interest of a limited number of workers for many years and notable contributions were made by some of the pioneers of experimental biology. Within the last decade, however, great advances have been made by a host of workers. In large part this has come about from two causes. On the one hand, for a number of reasons the control of many insects whose activities are of importance to agriculture is less satisfactory now than it was in former years and practical entomologists have come to realize that knowledge of the inner workings of insect life is essential for future advances in their control. On the other hand, the growing realization of the importance of insects as carriers of many diseases of man and animals has led to great interest from the viewpoint of medical and veterinary science. And many workers are interested in the subject for its own sake. In consequence, a very large literature has come into being. Arrangements for publication have not kept pace with production, with the result that papers having to do with insect physiology are appearing in scores of journals, many of which would not be expected to contain such material.

The present summary of recent work is limited entirely to the last five years. This has necessitated the discussion of many recent advances without mention of their background, or of the authors directly responsible for the initial studies, for which only the excuse of the necessity for brevity can be offered. For the same reason the term physiology is interpreted rather narrowly and the closely allied subjects of insect anatomy, pathology, embryology, evolution, ecology, toxicology and control and medical entomology are ignored, although their relation is often very close. Biochemistry is included as a part of physiology. A total of approximately twelve hundred papers has been considered, practically all of them in their original form, in selecting the four hundred and forty-four which are cited and listed in the bibliography. The aim has been to mention most of the fields in which active work is

going on and to choose for citation only leading articles, particularly those having comprehensive bibliographies. Critical analysis has in general not been attempted because the subject has too many parts for the personal acquaintance of any one or two workers.

For the guidance of the reader who is interested in learning more about certain subjects a number of recent reviews in addition to those cited in the text are included in the bibliography. Wigglesworth's *Insect Physiology* (427) is an authoritative exposition of certain subjects, to many of which that author has contributed. Imms' *Recent Advances in Entomology* (189) has several chapters on physiological subjects of which those on insect coloration and on parasitic relations are particularly valuable. A comprehensive discussion of the relation between insect physiology and insect toxicology has been given by Kusnezov (220). Uvarov (401) has written a valuable summary of work on insect nutrition up to 1929 and has given a very useful account of the relations between ecology and physiology (403). Many aspects of insect physiology and medical entomology are discussed in the symposium on bloodsucking and non-bloodsucking flies held at the 26th annual meeting of the Entomological Society of America (347).

A study of the geographical distribution of workers in insect physiology shows that they are chiefly located in Germany, the United States, England and the U. S. S. R. The increasing number of publications in the Russian language is particularly striking. It is also evident from an examination of references cited that Americans seem to be in the habit of ignoring work done in other countries. This is very regrettable and it is hoped that future workers in insect physiology will become acquainted with the labors of others in all parts of the world.

INSECT BODY AND PRODUCTS. *Composition of the insect body.* It has been known for a long time that, in general, the same elements occur in insects and in mammals though the proportions may be very different. Thus in Locustidae (402) the following elements have been reported:

TABLE 1

	ELEMENT										
	Na	K	Ca	Mg	Cu	Fe	Mn	Si	P	S	Cl
Per cent of live weight	0.16	0.52	0.15	0.11	0.005	0.05	0.006	0.21	0.49	0.035	0.014
Per cent of ash	4.6	15.0	4.4	3.0	0.13	1.4	0.16	5.9	14.0	1.0	0.4

The total phosphorus in several species of lepidopterous pupae averages 0.23 per cent of live weight and the inorganic fraction is nearly three times as high (0.083 per cent) during midwinter as it is in early fall. Adult females contain about twice as much inorganic phosphorus (0.053 per cent) as males (71).

Several investigators have been interested in the occurrence of heavy metals which may be important in respiratory or oxidative processes. Copper has been found to vary from 0.0014 to 0.0056 per cent of the live weight in ten species from the orders Orthoptera, Coleoptera, Lepidoptera and Diptera and to be eliminated in cast skins, egg cases and cocoons (259). It comprises 0.0008 per cent of the live weight of the American cockroach and 0.0006 per cent of the weight of the silkworm egg (328). A function in tissue oxidation has been attributed to copper. In several species of Coccidae (134) copper averages 0.032 per cent of the total weight and is lower in scales collected in regions where resistance to cyanide fumigation is prevalent.

The amount of manganese varies greatly in different insects. In the ash of certain of the Pentatomidae (Order Hemiptera) 0.47 per cent was found (402). Over forty species from six orders of insects (406) contained an average of 0.001 per cent manganese based on live weight but in one species of ant 0.14 per cent was present, largely concentrated in the posterior portion of the body where it may be concerned in the production of formic acid, which occurs in the poisonous liquid of the sting. On the other hand no manganese was found in the ash of the American cockroach (328). Iron occurs to the extent of 0.003 per cent in the cockroach and 0.004 per cent in the silkworm egg based upon live weight in each case (328). In red scale 0.05 per cent iron has been found, part of which is apparently in organic combination (134).

Organic constituents of the insect body usually have been studied in relation to some aspect of metabolism and will be considered in the discussion of the several physiological processes.

The integument. The physiology of insects is profoundly influenced by the presence of a more or less rigid exoskeleton, whose extent varies from sclerotization of only the mouth parts to practically complete envelopment of the body and its appendages. The exoskeleton is incapable of growth and the body therefore can increase in size only by smoothing out of wrinkles in the integument, which allows for limited expansion (425). Some parts of the body, notably the head capsule, cannot expand. At intervals during the growing period the integument is shed and before the new one can harden rapid increase in body size

occurs, the process often being aided by large volumes of air which the insect swallows for that purpose. This mode of growth divides the life of immature forms into instars and places each insect in a particularly helpless and vulnerable condition between the loss of the old covering and the hardening of the new one. A limitation upon size is also imposed because distortion on account of gravity after molting of a large unsupported body would lead to hardening in shapes not suited for the necessary activities of life. The peculiar tracheal mode of respiration probably also is connected with the exoskeleton since the functioning of lungs would be more hampered than that of a tracheal system within a rigid body covering. The attachment of muscles to an outer support can be shown on physical principles to be much more efficient than attachment to an inner skeleton and many of the feats of strength shown by some insects can be explained on this basis.

The per cent of chitin, expressed as chitosan, in the exoskeleton of the cockroach, *Periplaneta fuliginosa* is 29.6 per cent, and ranges from 37.65 per cent in the dorsal abdomen to 18.22 per cent in the hindwings. On the basis of live weight it is 2 per cent. No correlation exists between chitin content and hardness or pigmentation (377). This has been pointed out for other insects by earlier workers.

The total amount of "skeletal tissue" has been determined in several insects by removing all other parts of the body either by boiling in dilute alkali or by digesting with enzymes (60). The two methods agree in showing from 8 to 20 per cent insoluble matter. Such studies are of limited value, however, because the integument of insects is extremely complicated both chemically and physically. One of the most complete studies of the integument has been reported for the bloodsucking bug, *Rhodnius prolixus* (Triatomidae, Hemiptera) (425). Structurally two layers may be distinguished, an extremely thin, amber-colored epicuticle lying over a much thicker endocuticle which in the folds and other softer portions of the body consists of an unpigmented laminated layer. In rigid areas the endocuticle contains another layer just beneath the epicuticle called the exocuticle, which is usually colored and is responsible for the rigidity. Beneath the endocuticle is a single layer of cells from which, together with the glandular cells, all of the integument with its glands and processes are derived. Numerous ducts, whose function will be discussed under "Ecdysis," penetrate both layers of the endocuticle.

The endocuticle has been shown to contain both protein and chitin. The epicuticle contains neither, but apparently is a complex lipoid to

which the name "cuticulin" has been applied (425). This substance is undoubtedly responsible for the fact that most insects are not readily wet by water. Certain peculiarities in the respiration of aquatic forms are dependent upon this property as are many problems connected with the application of insecticidal sprays. Cuticulin is not elastic and the rigidity of the exocuticle is caused by infiltration of cuticulin.

Nymphs of *Rhodnius* can ingest from 6 to 12 times their weight of blood at a single meal because their endocuticle is free of cuticulin and their epicuticle is deeply folded. In the adults the exocuticle is impregnated with cuticulin and cannot be stretched, but expansion of the abdomen is possible by an entirely different mechanism, i.e., straightening of the folds in the body wall along the sides of the abdomen.

The ease of penetration of the integument by various substances is of great interest and some work and much speculation have been done on the relative importance of direct penetration through the integument as compared with entrance by means of the tracheae. Contrary to popular opinion chitin is neither impermeable nor rigid. This is illustrated by the anal gills of mosquito larvae which are covered only by chitin and are both elastic and highly permeable. Similar properties are shown by the peritrophic membrane of the intestine which is practically pure chitin. It is the extremely thin cuticulin of the epicuticle which is responsible for the comparatively low permeability of insect integument (427a).

The toxicity of a series of acids to mosquito larvae has been shown to run parallel with the lipoid solubility of the acid molecules as would be expected if a lipoid material controlled penetration (50). The same insects are readily penetrated by dissolved nicotine molecules (320) and molecular arsenious acid enters through the integument of mosquito pupae (181). Nicotine solutions placed upon the tips of the antennae or upon the wings of butterflies cause typical signs of nicotine poisoning (306). Pyrethrum or sodium fluoride produces toxic effects when placed upon cockroaches at a distance from the spiracles (142), (174). When portions of the bodies of corn earworm larvae, grasshoppers or cockroaches with spiracles sealed were exposed to the vapor of pyridine, piperidine, or nicotine the substances could be detected in the body within a short time and they were distributed throughout all the tissues (322). However, the integument is not a uniform sheet but is of varying thickness and contains a multitude of cells and glands of various kinds whose distribution is very uneven. Experiments such as those mentioned above do not distinguish between entrance through

glands and through the intact integument. Apparently no work on that subject has been reported.

Integumental glands. In addition to several kinds of sensory cells or glands which will be considered later in connection with sensory behavior there are a number of glands lying in or derived from the ectodermal cells which produce important substances. Silk is formed in modified salivary glands of many lepidopterous and trichopterous larvae and varies considerably in composition in different species (357). Lac is a mixture of an albumin, several polypeptids, a true fat and a wax (318). It is produced by the coccid, *Tachardia lacca*, and is synthesized in lac cells scattered in the integument. Other coccids produce wax also, e.g., the shell formed by the fluted or cottony cushion scale, *Icerya purchasi*, is almost entirely wax and is probably ceryl cerotate (267). From beeswax, which is formed in integumental glands of the abdomen, many fatty acids have been isolated, among which hydroxy palmitic is particularly interesting (188). Cellulose, lignin and several other complex carbohydrates occur in the shell of the Japanese coccid, *Cerococcus muratae*, but there is no chitin (209).

GROWTH AND METAMORPHOSIS. *Development of embryo.* Recent investigations of embryological development in several different kinds of insects have confirmed the usual ideas concerning the origin of the various organs. They have shown that the fore and hind guts are of ectodermal origin, derived respectively from the stomodeum and proctodeum but have not yet entirely settled the controversy regarding the existence and origin of the endodermal layer and, therefore, of the midgut. A very complete summary of work from the earliest time up to 1930 has been given by Eastham (89). From his work on *Pieris rapae* he has attempted to reconcile opposing ideas on the theory that the midgut is formed from two mesentoron rudiments which arise in the region of the future mouth and anus either before or after the beginning of the stomedeal and proctodeal invaginations and are carried toward each other at the tips of these invaginations as the latter deepen. Since in many insects these mesenteron rudiments can be clearly distinguished before there is an ectodermal invagination, there is a strong case against their origin being ectodermal (88). Recent work is consistent with this viewpoint. In the case of the closely related insect, *Pieris brassicae*, the ends of the stomodeum and proctodeum are considered to be blastopore lips and to give rise to endoderm. They persist into the adult as the imaginal rings of the gut during metamorphosis. The imaginal rings of the Malpighian tubules are parts of

the blastopore lips and thus the tubules are of endodermal origin (157). In the flour beetle, *Tribolium confusum*, the posterior mesenteron rudiment can be seen at a very early stage but the anterior one does not appear until the stomodeum has invaginated (176). Mesenteron rudiments closely connected with the stomodeum and proctodeum give rise to the endoderm in the black fly, *Simulium pictipes*. The development of the esophageal valve isolates a group of cells of ectodermal origin which seem to be the same as those which form the peritrophic membrane in the larva (122). The question of the origin of the midgut will be further considered in connection with the problem of what regions of the digestive tract are absorptive.

The embryo of the grasshopper, *Melanoplus differentialis*, about twelve days before hatching, is isotonic with a modified Ringer's solution containing 0.9 per cent NaCl, 0.02 per cent each of KCl, $CaCl_2$ and $NaHCO_3$. Variations in concentration of -10 per cent to $+30$ per cent cause no interruption in development, and adjustment after a time is possible with much greater variations in concentration (362).

An interesting study on the variation in sensitivity to x-rays during the embryological development of the blowfly, *Calliphora erythrocephala*, showed a general decrease in sensitivity as the period of incubation increased. There are two periods, however, when the sensitivity temporarily increases. These were proved to be the time when there is particularly intense cellular activity in the embryo (348). The eggs of *Drosophila* are most easily injured by alpha particles during cleavage and gastrulation and are most resistant during organogenesis (155). When most susceptible to alpha particles the eggs are most resistant to x-rays, which is explainable in terms of relative penetration and movements of the active cells.

Hatching of the egg. The process by which insect larvae escape from the egg has attracted attention for many years. A particularly detailed account has been given recently for six widely different insects (357). In each case the fully developed larva swallows the amniotic fluid followed by more or less air. Larvae of many insects, including the flea, *Ceratophyllus wickhami*, the bedbug, *Cimex lectularius*, and the sucking louse, *Polyplax serrata*, have hatching spines which tear through the vitelline membrane and the chorion. The larvae escape through the resultant hole. The latter two insects split, and escape from, the embryonic cuticle at the time of emerging from the egg. This is analogous to a first molt and they enlarge the body by swallowing more air before the integument hardens. The larva of the blowfly, *Lucilia sericata*,

pries off a flap at the head end of the chorion and with its strong mouth hooks ruptures the vitelline membrane which is stretched across the hole. The larva of the grain moth, *Sitotroga cerealella*, gnaws a hole at the head end and the larva of the mealworm, *Tenebrio molitor*, elongates the body forcibly and breaks off one or the other end of the egg shell. It also has an embryonic cuticle which is lost during hatching.

Growth of immature forms. The newly hatched insect seeks at once for food. Eating may be discontinuous as with the bloodsuckers or nearly without cessation as with those forms which suck plant juices, but eventually each young insect ceases feeding and goes through the act of ecdysis or shedding of the integument. The physiological processes underlying this action have received careful study in the case of a few insects.

Ecdysis. The nymph of the bedbug, *Cimex lectularius*, undergoes ecdysis after a full meal of blood, the interval after the meal averaging seven days for the first two instars, eight days for the third and fourth instars and nine days for the fifth instar. The period does not depend upon length of fast nor upon completeness of the meal, except that if it is too scanty, molting does not follow. In fact several partial meals may be taken within one instar, and one individual was given seventy-five small feedings before molting occurred (198). On the other hand larvae of the mealworm, *Tenebrio molitor*, may molt during starvation (393). The first step in molting of the silkworm larva is the formation of an inner layer of chitin from the secretion of certain hypodermal cells. The molting fluid is then produced by other hypodermal structures called exuvial glands and spreads between the old and new cuticle until all portions of the old outer covering are loosened. It is said to be mostly swallowed before the actual separation from the old covering is started (410). The molting fluid was once considered to have an excretory function because crystals of uric acid were found on the new cuticle of the silkworm, but this is a contamination from the Malpighian tubes, and if they are removed previous to molting, the crystals do not occur (351). The molting fluid has been attributed to the epithelial cells in the case of lepidopterous larvae (52) and of certain Orthoptera, Diptera, Coleoptera, and Hymenoptera (180).

A very complete investigation of ecdysis in nymphs of the bug, *Rhodnius prolixus*, has established the functions of the various cells and glands during the process (425). Molting occurs on the average fifteen days after the meal during the fourth instar and twenty-eight days after feeding during the fifth instar. In either case, shortly after feed-

ing, the epidermal cells become active and increase in number. Within a few days the new epicuticle can be found as an exceedingly thin membrane between the epidermal cells and the endocuticle. It forms in many folds which allow for expansion of the insect after molting. About this time the dermal glands (Verson's) become active and produce the molting fluid. This is a neutral liquid, free of chloride but containing protein. It dissolves the endocuticle and, therefore, contains a protease and a chitinase but does not attack the cuticulin which makes up all of the old epicuticle and part of the exocuticle. Most of the old cuticle is dissolved and is reabsorbed so that only a small fraction is eventually lost. At the same time the molting fluid is almost entirely reabsorbed. This occurs through the new epicuticle which must be much more permeable than it is later. A third kind of cell, the oenocytes of the epithelial layer, become active soon after the new epicuticle is formed. It is possible that they secrete the non-chitinous portion of the cuticle. This is the more probable because they are also active during maturation of ova in the female when the egg shells are being formed.

The actual casting of the old cuticle is brought about by the insect swallowing air until the pressure is increased sufficiently to split the remnant of the old covering along some line of weakness. After emergence more air is usually swallowed so that the new cuticle is expanded. It is usually pliable and colorless at first. Exposure to the air soon leads to stiffening and the development of color but physiological factors are also concerned for if the cuticle is stripped from a portion of the body prior to molting the new cuticle remains soft and pink until after molting occurs. If bedbug larvae are killed immediately after molting their cuticle does not darken or become hard (198). It is likely that the effect of internal physiological processes is transmitted to the epicuticle by way of the pore canals that extend through the exocuticle and the endocuticle (424).

The physiological factors which initiate ecdysis have received attention in the case of only a few insects. Growth and the resultant internal pressure upon the cuticle are probably of importance, but a substance present in the blood at the time of molting is undoubtedly of most significance. A group of butterfly caterpillars into whom blood is injected from other molting caterpillars molt sooner than a similar group injected with blood from others not near molting (53). In this case it was concluded that the effect was produced by a hormone elaborated in Verson's glands. Experiments of the same nature with the bug,

Rhodnius, showed that there is a critical time in each instar, e.g., seven days after a meal in the fifth instar, which corresponds with the time when mitotic division of the epidermal cells begins, after which the blood has the ability to stimulate molting in another individual (427). Removal of the head before this critical time prevents molting of the headless bug, but beheading at a later time has no effect upon the process. The only organ in the head which undergoes a cycle of activity during the critical period is the corpus allatum, which therefore is considered to produce the molting hormone in this insect.

Additional confirmation of the hypothesis of hormone control of ecdysis is given by experiments in which legs of butterfly larvae were transplanted. The transplanted member molted at the same time as its new body even though that was of a different species and it even underwent fewer or more than the normal number of molts when the individuals were in different instars at the time of operation (28).

Number of instars. The number of immature stages is affected to a certain extent by external conditions. The grasshopper, *Melanoplus mexicanus*, reared at 22–27°C. has six instars but only five if reared at 32–37°C. (298). Likewise a higher temperature decreases the number of instars in the case of the butterfly, *Pieris brassicae*, which goes through five stages at 14–15° but only three at 22–27°C. (201). But larvae of the Mediterranean flour moth, *Ephestia kuhniella*, give the opposite response, the majority molting five times at 25°C. and four times at 18°C. (124). The number of larval instars of the Indian meal moth, *Plodia interpunctella*, varies from five to seven and is dependent upon how great an increase in size occurs at ecdysis, for those which increase but slightly pass through more instars. Those larvae of the black cutworm, *Agrotis ypsilon*, which pass through seven instars consume approximately ten per cent more food than those which go through only six stages, though the final size attained is about the same (341).

Rate of growth. The growth of insects has long attracted attention and investigations have been made on many conditions which affect it. The relative increase in weight or body dimensions during successive instars has been studied in connection with various hypotheses concerning the relation between growth and ecdysis. Dyar's rule which originally stated that the width of the head capsule of lepidopterous larvae increases in a geometrical progression at each instar has been found to hold for sawfly larvae, *Phyllotoma nemorata*, if average widths are used, and may be used to decide upon the number of instars. But

individuals vary greatly during development (378). Also the rule applies only to feeding stages and no increase occurs during the ecdysis before the prepupal instar which is non-feeding. Male larvae of the gypsy moth, *Porthetria dispar*, follow the rule, the factor of increase averaging 1.6, but female larvae vary widely (197). The progression factor decreases with each instar of the bedbug larva (394) and of the silkworm, the races of which differ somewhat in this respect (63). The contrary condition is shown by nymphs of the grasshopper, *Melanoplus differentialis*, which undergo relatively greater increase in weight during the latter instars (175).

Other studies of relative larval growth have dealt with the theory due to Przibram that during each instar all the cells of the body divide (313). This seems improbable for it has been shown that in insects, as in other animals and in plants, the various parts of the body grow at different relative rates at different periods of development and cells may increase in size without dividing. Applied to a single organ, the rule is much more likely to hold. According to this, if the head capsule grows in proportion to the rest of the body, the coefficient for Dyar's rule should be $\sqrt[3]{2} = 1.26$, which is somewhat lower than usually has been reported. During most of the nymphal stages of the mantis, *Sphodromantis bioculata*, the facets of the compound eyes increase in length by the factor 1.26, i.e., their growth in this dimension illustrates the rule. In several other insects, however, growth of the compound eye facets corresponds more closely to a doubling of the *area* in each instar (312). The increase in weight of individual larvae of the Japanese beetle, *Popillia japonica*, agrees well with the theory of doubling the number of cells in each instar, but averages of groups of larvae give results at wide variance. This is interpreted as probably due to the known variation in number of instars which is six or seven and occasionally eight (231). An extension of the idea of a variable number of instars has been made in the case of a considerable number of insects whose larval growth did not conform to Przibram's theory, to the effect that they undergo several "latent" molts. According to this idea most holometabolous insects, i.e., those undergoing a complete metamorphosis, pass through twelve or more larval instars (23) (24).

Variations in the relative rates of growth of various parts of the body have been investigated in connection with the equation for differential growth ratio, $y = b\,x^k$ in which y is the size, weight, etc., of one organ, x is some property of another organ or of the whole body, and b and k are constants. This signifies that the logarithm of the magnitude of

one part is proportional to the magnitude of the other. If $k = 1$, the two processes which are being compared are isogonic, i.e., they are taking place at rates which have a constant ratio. If $k \gtrless 1$, the processes are heterogonic and one or the other is occurring at an accelerating rate. The length of the mandibles of stag beetles (Lucanidae) has been shown to be heterogonic with respect to total length and it is concluded that a number of forms of male lucanids differentiated by systematists really differ only in the heterogony of their mandible growth (186). Many growth processes in the stag beetle, *Lucanus cervus*, the stick insect, *Dixippus morosus*, the mealworm, *Tenebrio molitor*, and the wax moth larva, *Galleria mellonella*, have been shown to be heterogonous, in fact it is the exception to find two processes that are isogonic (187). Application of the principle of heterogony has been made to many metabolic processes such as total water in the body, total nitrogen, total fat, ash, etc. (393).

Temperature and growth. Of all the factors concerned with the growth of insects, temperature has undoubtedly received the most attention, though nutrition seems likely to take the first place soon. These investigations have been of the most diverse character but may be roughly grouped into three classes: *a*, those having to do with the length of a developmental period, of adult life, or of the entire life at one or at various temperatures; *b*, mathematical analysis of the effect of temperature upon rate of development; and *c*, those concerned with the upper and lower temperatures at which development is possible. Humidity is a complicating factor whose influence often cannot be isolated from that of temperature.

The rate of development of a number of important insects has been studied recently without any attempt to formulate mathematical relations from the observations. Thus the time for hatching of the egg of the mosquito, *Culex pipiens*, is 9 to 11 days at 10°C., 2 to 3 days at 15°C., but only 24 hours at 20°C. (37). The larval stage of the human louse, *Pediculus humanus*, is (in days) 22 to 23 at 23°C., 20 at 27°C., 15 to 16 at 30°C., 11 to 12 at 34° C., 10 at 37°C., and 12 to 13 at 40°C. (9). The total time required for larval development of the bedbug, *Cimex lectularius*, is 326 hours at 32°C., 414 hours at 27°C., and 855 hours at 22°C. Variation of relative humidity from 10 to 90 per cent had little effect (327). The larval periods of the gypsy moth, *Porthetria dispar*, vary greatly with temperature, e.g., at 100 per cent relative humidity the length of the first instar is 31 days at 10°C., 8 days at 20°C. and 6.5 days at 28°C. Ten per cent reduction of the relative

humidity at each temperature prolongs the period to 50, 11 and 7.5 days respectively (5). With the grasshopper, *Melanoplus mexicanus*, the egg stage is 147 days at 22°C., 26 days at 27°C., 32 days at 32°C., and 46 days at 37°C. when the eggs were kept in moist sand. At 27°C. relative humidity below 30 per cent prevented hatching and up to 70 per cent was increasingly favorable and from there to 100 per cent equally favorable. At higher temperatures, 32° and 37°C., successively greater relative humidity was essential for hatching to occur (298). A study of the length of life of adult *Anopheles subpictus* mosquitoes indicates that under the climatic conditions of the Punjab the females do not live long enough for the malarial parasite to complete its development, which agrees with the observation that it is not a vector (258). This same condition was noticed in California in connection with the mosquito, *Anopheles maculipennis* (115).

It will be noticed from the above examples that the rate of development rises to a maximum at some intermediate temperature and then decreases. Hence the maximum velocity of a process under consideration occurs at some temperature often called the "optimum temperature." Similarly, there is usually an "optimum humidity" for development. It by no means follows that these conditions are most favorable for the insect, for maximum rate of growth is not necessarily associated with maximum vigor or reproductivity. The other point of importance is that over a range, which varies greatly with different insects, there is a direct variation of rate of development with temperature, and sometimes with humidity (8). Most of the relations which have been suggested in connection with the growth of other forms of life have been applied to insects (194). The simple relation that the product of temperature and time required for development is a constant has been used often and may be illustrated by the case of the incubation period of the egg of the flesh fly, *Lucilia sericata*. The expression for developmental time for temperatures of 14°C. and above is $T(t - 9.5) = 8.5$ in which T is time in days and t is Centigrade temperature (411). It follows that at a temperature of 9.5°C. an infinite time would be required, i.e., development would be completely halted. The constant 8.5 is in day-degrees and is known as the "thermal constant," and, of course, may be expressed in other units. It differs widely for different insects and for various stages of the same insect, being influenced both by the slowness of the process and by its limiting lower temperature. It has long been recognized that a "developmental threshold" calculated by downward extrapolation often leads to erroneous results.

Recent work has afforded further examples of the fact that development actually proceeds at temperatures often considerably lower than the calculated ones, i.e., there is deviation in a positive sense at low temperatures. The opposite condition is shown above the temperature for most rapid development for beyond this point negative deviation occurs and, indeed, fatal conditions usually are reached soon.

An example of the error involved in determining developmental thresholds by extrapolation is given by the flesh fly egg previously mentioned. Instead of 9.5°C. it is actually about 5.5°C. (411). The limiting temperature for pupal development of the codling moth as calculated by downward extrapolation is 10°C., but experimentally it is 2 to 3°C. (349).

In attempts to represent conditions at extremes of temperature more accurately a number of mathematical relations have been used. Van't Hoff's rule that velocities at temperatures ten degrees apart have a constant ratio holds fairly well over the median range where it gives nearly a linear relation between temperatures and rates of development, but it actually deviates in the wrong direction at both extremes (403c). An exponential curve of the double catenary type has been recommended because it approximates the actual deviations at both ends of the developmental range (8) (194) (265). Length of adult life is also influenced by temperature and in one case, that of the bean weevil, *Bruchus obtectus*, is well represented by the Van't Hoff rule since it correctly represents the accelerating effect of high temperature upon death rate (265).

Reports on temperature limits for development are very numerous, but such data by themselves are ordinarily of little importance for if continued long enough, zero rate of development can only result in death. Hence what is usually determined is the survival time at certain temperatures at either end of the developmental range. This subject of limiting conditions for life will be considered later.

Under natural conditions insects are practically never at a constant temperature. For this reason attention was directed many years ago to the effect of varying temperature upon insect growth. Recent work has thrown light on certain contradictory findings of earlier workers. Exposure to temperatures above the median range usually retards development, probably due to actual injury although when exposure occurs early in development, the effects may not be noticeable until much later (193). Alternation over short intervals within the median range usually has no accelerating or retarding effect. After development has begun, alternate exposure to low and median temperatures

has two different effects depending upon whether the insect is accustomed to spend the cold season in a dormant condition. Larvae of the mosquito, *Anopheles plumbeus*, hatched in the fall, develop faster at outdoor temperatures averaging 16°C. than they do indoors at 21°C. (332). Such larvae customarily hibernate and their behavior is connected with the phenomenon of diapause which will be discussed later. On the other hand, insects which do not undergo seasonal dormancy are retarded by alternation between median and low temperatures, e.g., hatching of bedbug eggs is delayed (143).

Pupation. The last process occurring before the adult insect appears involves a more profound change in form than any that take place during molting of immature forms. In the case of the endopterygotan insects a more or less complete reorganization occurs during the pupal stage and even with exopterygotan forms a rather extensive change may occur without the intervention of a separate stage during which this takes place. The processes are analogous and differ only in extent of the reorganization. With the first group the pupal stage may be considered to consist of an initial period during which the predominant process is histolysis or destruction of larval tissue and a succeeding period of histogenesis or organization of adult tissue. As might be expected, marked changes in chemical constitution occur. With the blowfly, *Lucilia sericata*, the insoluble protein decreases and soluble protein and split products increase throughout the first stage, followed by the reverse change during formation of the adult. Excretory nitrogenous products are formed almost solely during histolysis. Fatty acid content decreases during most of the pupal period but only unsaturated acids are used up (95). In the grain beetle, *Tenebrio molitor*, histolysis and histogenesis are not so distinctly marked and hence changes in composition are much less abrupt (97). Studies of changes in blood composition during pupation of the moth, *Deilephila euphorbiae*, have shown it to change much more than the solid tissues and also that about 70 per cent of the energy needed during this period of metamorphosis is derived from changes in the blood (153). The pH of the blood falls from about pH 7.1 to 6.8 during the late prepupal period of the Japanese beetle (232). Likewise the pH remains constant during the first half of the pupal period of the bee moth, *Galleria mellonella*, and falls rapidly in the second half, parallelling the production of carbon dioxide (379). This is of considerable interest, for histolysis has proceeded considerably before this change occurs and it is, therefore, likely that the change in pH is a consequence and not a cause of histolysis.

The histological changes involved in the final metamorphosis of a

number of insects have been shown to be extremely varied. Thus the cells of the midgut of the honeybee larva are cast into the lumen and replaced from the imaginal or "replacement" cells which have remained dormant up to this time (293), while in the rice weevil, *Calandra oryzae*, the midgut is almost entirely replaced from buds attached to the foregut and the local imaginal cells have little part in its formation (281). On the other hand strictly adult tissue seems always to be formed from the imaginal discs or plates, e.g., leg muscles of holometabolous insects.

The process for the destruction of those larval cells which are not reorganized directly into imaginal tissue varies in different insects. Phagocytosis is the predominant process in Diptera but in most orders the cells seem to dissolve and allow their contents to disperse. It has been suggested that the phagocytic cells of Diptera correspond to the myoblasts of imaginal cells dormant in larval life of Hymenoptera (293). In any event it seems clear that degeneration of larval cells is extensive even in those cases in which they are finally destroyed by phagocytosis. In the case of the rice weevil this condition has been connected with hypertrophy which larval cells undergo just before metamorphosis and which the dormant imaginal cells escape (281).

The many hypotheses which from time to time have been advanced to explain the initiation of metamorphosis (427e) are now largely replaced by the idea of hormone control. This hormone is different from the one which causes molting (28). This has been illustrated with the bug, *Rhodnius*. If a nymph of any of the first four instars is decapitated soon after feeding and then injected with blood from a metamorphosing fifth instar nymph it develops adult characteristics instead of the normal nymphal ones. Conversely, if fifth instar nymphs decapitated soon after a meal receive blood from molting fourth instar nymphs they molt instead of becoming adults (427). Another hormone is hypothecated to explain the inhibition for metamorphosis prior to the last molt. The head is necessary for the production of this substance for if early instar nymphs are beheaded during a certain critical period in each instar they molt but develop adult characteristics (427). The validity of this interpretation in the case of other insects apparently has not been tested.

Abnormalities. Exposure of immature insects to unusual conditions may lead to the production of monstrosities during metamorphosis. Similarly transplantation sometimes leads to peculiar abnormalities as in the case of the butterfly, *Vanessa urticae*. Grafting of the anlagen of larval legs on to larvae causes duplication in the adult of the claw

and sometimes of the tarsus but not of the whole leg (27). Forms intermediate between larva and adult may be produced by the action of low temperature on larvae of the mealworm, *Tenebrio molitor* (283) or by x-rays on larvae of flesh flies (185) or of *Drosophila* (346). Such forms sometimes occur without exposure to abnormal conditions, e.g., one in ten thousand Mexican bean beetle larvae have wing pads (223) and wireworm (Elateridae) pupae with certain larval characteristics occur in nature (383). The explanation of such abnormalities is as yet uncertain, but probably they are due to the irregular effect of the hormones previously mentioned in connection with the processes of molting and metamorphosis (427f).

Regeneration. Adult insects of most orders are not able to regenerate lost parts of the body. However, when molting occurs in the adult stage, as among the Thysanura (bristle-tails), the antennae, cerci, and distal parts of the legs are capable of regeneration (372). Regeneration apparently is dependent upon the epidermal cells, e.g., those lying near the base of a bristle of butterfly larvae are responsible for regeneration of the bristle (26). Amputation of bristles did not affect rate of development of this insect. Restoration of the leg of the stick insect, *Dixippus morosus*, may come about in two ways: *a*, with autotomy, i.e., the injured portion is dropped off at the next joint during the next molt, or *b*, the lost parts are regenerated upon the stump. The order of restoration is: nerves and tracheae, muscles and sense organs (116). Regeneration is altered by transplantation of another appendage. In the case of the walking stick insect, grafting of the proximal joint of an antenna upon the coxa stump of a front leg results in regeneration of the antenna but no regeneration of the leg, whereas the leg is restored if no grafting is done (29, 30). Regeneration of leg or of antenna is not dependent upon presence of the corresponding ganglion but appendages developed after extirpation of their ganglia are not innervated (370). The Johnston organ, which occurs within the second antennal joint has nothing to do with regeneration of the antenna of *Dixippus morosus* or of the Mantid, *Sphodromantis bioculata* (120). The period at which parts are removed or damaged has a great effect upon the degree of recovery for if done in the pupal stage only slight changes, e.g., in the color patterns or size of butterfly wings can be caused without complete loss of the part or death of the insect (103). Damage to the wing buds in the last instar results in defective wings, but at an earlier instar complete regeneration is possible (245).

Sexual development. The development of the sexual organs has re-

ceived attention by recent workers. In the flour beetle, *Tribolium confusum*, germ cells are separated from the somatic cells during gastrulation and may be distinguished at that stage by their large size and granular chromatin. Differentiation of male and female sex organs occurs in the late embryo stage. This means that any change in sex ratio caused by treatment of the larvae involves an actual sex reversal (176), (178). In the black-fly, *Simulium pictipes*, germ cells appear early in the blastoderm at the posterior end of the egg. They remain in two groups on either side of the posterior portion of the body and are eventually carried forward to a level with the developing Malpighian tubules (122).

The embryo of the grasshopper, *Melanoplus differentialis*, develops but little during the winter. Rapid development of embryonic rudiments and distinction of the sexes follow revolution of the embryo in the spring (285). On the basis of observations on the grasshopper embryo a general theory of sex cell differentiation has been elaborated (286). Sexual maturity in the female migratory locust, *Locusta migratoria*, is not reached until copulation takes place (310). During the first period of growth in the ovary the egg of the cockroach is nourished only through the portion of its surface which is in contact with special nutrition cells. The remaining surface is impermeable to salts, acids, alkalis, dyes and hydrogen peroxide but is permeable to water. During the second period all parts become freely permeable (266).

In the majority of insects, hatching of the egg is dependent upon its previous fertilization by sperm. Exposure of the fertilized egg to unfavorable ecological conditions may prevent hatching. Similar exposure of adults may cause them to produce sterile eggs or sperm. Unfertilized females of the grasshopper, *Melanoplus differentialis*, lay eggs as readily as fertilized ones and embryonic development proceeds for a time at the normal rate (200). Such developing cells may be either haploid or diploid and the change to the diploid condition does not occur at a uniform period. Such unfertilized eggs rarely produce normal adults and deformed embryos are the rule. Butterflies which do not feed have some or all of their eggs fully developed upon emergence of the adult, but in those which feed and live longer much of the egg development occurs during adult life (91). Ultra violet light and x-rays applied to females of the bean weevil, *Bruchus obtectus*, and of the moth, *Callosamia promethea*, cause sterility of their eggs. Eggs treated after laying are killed and larvae are killed or prevented from pupating normally. Young pupae seem to be much more resistant and fertility

of the resulting adults is not affected in either sex, but exposure just before emergence of the adult results in sterility (244), (145).

Determination of sex. The sex ratio varies very widely in different groups of insects. It is of particular importance as a factor in the reproductive capacity and in the population level attained under given conditions. Differences in the sex ratio correlated with the abundance of food during the larval period have been noted by many workers but such effects may have been due to differential mortality of the sexes under unfavorable conditions. Thus, the percentage of females of the braconid, *Alysia manducator*, reared from blowfly larvae is directly proportional to the size of the larvae in which they feed (177). If newly hatched larvae of the flour beetle, *Tribolium confusum*, are starved for two to three days the percentage of females is increased and selective mortality is not considered to be responsible (178). This is contrary to the usual observation on the effect of adverse conditions and also means that sex may be determined during larval life. On the other hand starvation of *Lucilia sericata* (blowfly) larvae does not result in change of sex (243).

A very striking sexual phenomenon follows parasitism of the larvae of certain insects by *Stylops* sp. (Strepsiptera). This is particularly marked in several species of Homoptera and Hymenoptera. The hosts are able to pupate but often transform into adults which show alterations in structure and color sometimes extending to the sexual characteristics. The result is an individual which is partly male and partly female. In the case of certain wasps the amount of food available to the larvae determines whether this condition of intersexuality will follow parasitism. Those species for which the mother provides a limited amount of food at the time when the eggs are laid are prone to "stylopization" but those which are given as much as they can eat, while subject to parasitism, seem immune from these peculiar consequences (340).

The condition of intersexuality is not dependent upon parasitism, but occurs spontaneously in a wide variety of insects. Its occurrence in the gypsy moth has been extensively studied and a mechanism suggested which is partly genetic and partly physiological (128). A comprehensive review of his many years' work has been given by Goldschmidt (127). According to this theory each individual begins development with certain genes for maleness and certain ones for femaleness. In the great majority of cases one group is below a certain threshold value and hence the other is able to set in motion a series of physiological processes which result in an individual of one sex or the other.

But certain combinations of strong and weak races, as regards sexual genes, result in indecisive ratios. Two series of physiological processes are accordingly started which in general proceed at different rates. Accordingly it is possible for an individual who started as of one sex to continue to develop without change of sex, or to change at a late period or at an early period. Early change results in a high degree of intersexuality and late change in a low degree (125) which agrees with the embryological facts that organs differentiated early in development are changed but slightly in intersexuality whereas those which differentiate late are changed greatly. A detailed study has been made of the changes which ensue when a testis is changed into an ovary and vice versa. Hormones which affect the gonads but not the rest of the body are hypothecated (126). Temperature has an effect upon the occurrence of intersexual forms, e.g., with *Habrobracon juglandis* (parasitic Hymenoptera) more mosaic intersexes occur at 35–37°C. than at other temperatures (129). Sex determination in insects is being studied more from the viewpoint of genetics than from that of physiology. An excellent summary on *Drosophila* has been given by Bridges (46).

Oviposition. The factors which affect oviposition are both physiological and ecological. Unfortunately, practically no data have been reported on the first aspect though it is true in many species of insects that copulation provides a necessary stimulus. The epidermal cells in females of the blood sucking bug, *Rhodnius prolixus,* become active during egg laying and may have a function in forming the eggshell (425). Temperature and humidity as factors in oviposition have been widely studied. Certain recent reports show that there are optimum and limiting conditions for each species studied. The granary weevil, *Calandra granaria,* lays only between 13°C. and 35°C. and prefers a high relative humidity (397). The alfalfa weevil, *Hypera postica,* will not lay above 28°C. but any relative humidity from 50 to 95 per cent is satisfactory (371). The queen bee lays between 23° and 34.5°C. but prefers about 32°C. (87). The mosquito, *Anopheles maculipennis,* prefers 22° to 29°C. with a maximum range from 15° to 32°C., while *A. bifurcatus* prefers 12° to 20°C. and will not lay at 25°C. On the other hand *Aëdes aegypti* prefers temperatures from 20° to 30°C. (148). It is obvious that such temperature limitations will exert a very great effect upon the distribution of the various species.

Mosquitoes also have limitations regarding the pH of the water in which their eggs are laid. *Culex pipiens* will not lay in strongly acid waters—pH less than 4.4. It is interesting to note that this limit coin-

cides with the acidity which young larvae can endure (50). The preferred pH is 7.9 to 8.6 (4).

A rôle in oviposition has often been attributed to light, but clear cut results are few. The codling moth lays readily at a white light intensity of 10 foot-candles but is probably deterred at 30 foot-candles. The violet end of the spectrum appears to be more conducive to egg laying than the red end (146).

The number of eggs laid by a female varies enormously in different species of insects and the maintenance of the normal rate of oviposition is a very important factor in regulating the abundance of each species. Many observations are on record as to the number of eggs laid per day or during the life of an individual, but of chief physiological interest are data on the factors affecting the number. In *Drosophila* underfeeding reduces egg production, low temperature during development of the insect hastens egg production after the temperature is raised and number of eggs laid per day varies inversely as length of life (6). With the same insect it has been claimed that crowding has an immediate effect in reducing the number of eggs laid (301). The number of eggs that a blowfly produces is dependent upon its size and hence upon the amount of food consumed as a larva. One female *Lucilia sericata* lived 94 days and laid the exceptional number of 3171 eggs from which 2724 adults were reared (243).

Some recent data are available on the effect of temperature upon the egg production of certain insects. The interval 27° to 37°C. is suitable for maximum egg laying by the grasshopper *Melanoplus mexicanus*. The clear winged grasshopper, *Camnula pellucida*, has a somewhat narrower optimum range. The production of 15 egg masses by one female *M. mexicanus* is far above the two masses formerly thought to be the usual number (298). Temperature of 15°C. or lower prevents development of the ovaries in the sugar beet webworm, *Taxostege staeticalis* (444).

Reproduction in insects is usually sexual and by means of eggs, but parthenogenesis and viviparity are by no means rare. An interesting example of the latter is furnished by the tsetse fly which retains the larva within the uterine cavity until it is fully developed. Nourishment is provided by special "milk glands." In certain parasitic insects one egg gives rise to several hundred larvae. A limited number of cases of larval reproduction, or pedogenesis, have been found. All these peculiarities in reproduction are of great interest but, unfortunately, the physiological factors which bring them about, sometimes

regularly and sometimes sporadically, are not understood. They furnish some of the more important problems of the future.

NUTRITION. The nutrition of insects has received a great deal of attention recently. This has come about not only because the insects offer an extremely great variety of problems in this field but because the population level reached by many species, and hence their economic importance, is intimately connected with the kind of food which they can use.

Type and choice of food. It has long been considered that many insects that live in the water, such as mosquito larvae, derive much of their nourishment from the algal and plankton organisms which are always present in natural waters. However, it may happen that no relation exists between the abundance of such organisms in pools and their suitability for mosquito breeding. Accordingly their importance has been doubted and certain species of *Anopheles* and *Culex* mosquitoes have been reared on pool and lake water which had been passed through a Berkefeld filter (256), (257), (165). Hence the soluble organic matter provided satisfactory and sufficient foodstuffs. On the other hand anopheline larvae lived only six days and made but slight growth in dialyzed water from their normal breeding pools. This indicates that colloidal material is very important (167). A study of the filtering ability of *Anopheles maculipennis* larvae showed that they can select particles 20 mμ in diameter but not those one-fourth that size. A fourth instar larva was found to filter about 100 mm.^{3} of water a day (351).

Insects differ very greatly in the range of their foodstuffs, some such as the cockroach eating almost any kind of organic matter while others are very closely restricted to a particular kind of plant or animal. In fact, host preference has been used in segregating many species into "races" as will be discussed later. Opposition has been raised to this concept of strict host specificity and emphasis laid on the need for an adequate environment irrespective of whether it is natural or artificial (15). An extreme example is offered by several of the Hemiptera which are naturally plant feeders but under the impulse of hunger can subsist upon blood (399). It is probably a similar adaptability in the past which has brought about the present great diversity of food habits in this order. The contrary condition is shown by adult tsetse flies which must have blood and are poisoned by plant juices (141). An extremely interesting case of food choice is afforded by the fleshfly larvae which are used in persistent sores, long standing osteomyelitis, etc. Their

usefulness depends very largely upon their ability and inclination to consume necrotic tissue and to leave healthy tissue uninjured (13). Contradictory claims have been made concerning the behavior of *Lucilia sericata* and it has been stated and disputed that geographical races of this species act differently as regards normal tissue (48), (366).

Frequency and amount of feeding. The amount of food consumed by an insect is often the most direct measure of its economic importance. A study of several locusts which frequently are pests in Russia showed that an adult locust will eat food equal to one-third to one-half of its body weight every day and the total amount eaten during life is fully twenty times the adult weight (336). A very different insect, the blood-sucking bedbug, *Cimex lectularius*, consumes successively greater meals at each instar but the ratio of weight of meal to weight of body varies from about 7 in the first instar to 3 in the fifth. During its whole life the blood consumed is over six times the adult body weight. Females eat fully one-third more than males (195). The adult tsetse fly, *Glossina swynnertoni*, has definite hunger cycles every 5 days during which period it emerges from hiding in the brush and attacks man and animals (191). Further studies of the same and other species of *Glossina* have shown the length of the hunger cycle to vary with the time of year, the temperature and the humidity (192). Thus, *Glossina tachinoides* and *G. submorsitans* kept at 30°C. will not feed when the relative humidity is below 11 or above 88 per cent (62).

Temperature also has a marked effect upon the amount of food eaten each day by nymphs of the grasshopper, *Melanoplus mexicanus*, but the nymphal periods are likewise affected and the total amount eaten during growth is the same regardless of the temperature (297). A very peculiar effect of ultra violet radiation has been observed with the bean weevil, *Bruchus obtectus*, for irradiated larvae consume excessive amounts of food without corresponding increase in adult body weight (244). It seems likely that the smaller population attained by the flour beetle, *Tenebrio molitor*, in "conditioned" flour—i.e., flour in which they have lived for a protracted period—is due to distaste for such contaminated or altered food (296).

Requirements for growth and the rôle of microörganisms. For many years all consideration of the nutritional requirements of insects was complicated by uncertainty as to the part played by bacteria and other microscopic forms of life. These are present in nearly all insect food and specialized kinds are found as symbionts in the digestive tract of many insects. Leaving out of consideration, for the moment, the

effects of such symbionts, it has been shown in numerous recent investigations that insects resemble mammals in having definite needs for the various kinds of foodstuffs, including minerals and vitamins and that when these are furnished, the processes of life can be carried out on sterile food. Thus the larvae from sterilized eggs of the flesh fly, *Lucilia sericata*, grow rapidly and produce normal fertile adults on a sterile diet of casein, salts, butter, and dried yeast (270), and many workers have reared this insect and other flesh flies on sterile meat or mammalian organ tissues. On autoclaved meat the larvae of *Phormia regina* develop to the second or third instar and then die, but if the meat is sterilized by heating to 75°C. on four successive days normal growth results (109). This indicates the need for a heat-labile growth factor. Beef muscle extracted with water is inadequate for growth but yeast extract autoclaved without addition of alkali makes up the deficiency whereas autoclaved alkalized yeast extract is of no value (173). These results are strongly suggestive of one of the vitamin B group. Larvae reared on fatigued frog muscle are somewhat larger and their rate of heart beat is faster than those reared on resting frog muscle. The nature of the growth stimulant has not been studied (110). Sterile blood is not an adequate food for fleshfly larvae but it is made suitable by addition of a growth factor which cannot be extracted from muscle or from yeast by water. If the yeast is autolyzed, this factor becomes water soluble. On the basis of varying stability to heat and alkali, it is concluded that two heat stable growth factors are required in addition to vitamin B by fleshfly larvae (173). These substances are all supplied by the bacteria normally present in the food of the larvae. Results soon to be published from this laboratory show that vitamin A plays an important part in the pupation of *Lucilia sericata* larvae but that vitamins C and D are unnecessary.

In addition to serving as a source of growth promoting substances, bacteria perform the important function of liquefying food for fleshfly larvae. On sterile diets it is often found that growth is retarded just after hatching, but proceeds normally as soon as some of the food is liquefied. An interesting example of this is afforded by the growth of larvae on living sheep. Bacterial action is very beneficial because the infected tissues produce an exudate on which the young larvae grow excellently (242).

The omnivorous feeder, *Blattella germanica* (cockroach), fails to grow upon a diet of starch, cellulose, lard, sugar, salt and casein, but develops normally if cod liver oil and yeast are added. Nevertheless, it was

concluded that neither vitamin A nor D is needed. Among three proteins studied, casein was adequate, gelatin less satisfactory, and zein very poor, which indicates that there is need for particular amino acids (235). Similarly, the incomplete proteins do not suffice for fleshfly larvae (270). Attempts to supply the nitrogen needs of termites with inorganic nitrates and ammonium compounds were unsuccessful (328). They can live and grow on a diet containing sugar, protein, salts and vitamins A, B, D and G (70).

Another group of insects whose nutritional requirements for growth have been carefully investigated is the mosquitoes. Autoclaving the pool water in which they were breeding renders it unfit for growth of culicine larvae and reinoculation with various kinds of bacteria gives varied results (165). Neither excretory products nor extracts from bacteria render such sterilized water adequate but there is a substance within bacteria which stimulates the growth of these mosquito larvae. It is heat-labile, filterable, non-dialyzable and not readily soluble in alcohol, ether nor physiological saline solution (168). On the other hand, it has been concluded that bacteria do not furnish growth-promoting factors but are of value only because they are food for the larvae (335).

It usually has been considered that the roll of symbiotic microörganisms which occur within the digestive tract of insects or in adjacent mycetomes is to supply either an essential dietary requirement, such as a vitamin, or a digestive enzyme. Sterile blood is an inadequate food and those insects which subsist upon it alone throughout their life always have symbionts associated with the alimentary canal (10), (11). But symbiotic bacteria also occur in the mallophagans which do not suck blood but live entirely upon feathers and dead skin (322). *Rickettsia* microörganisms occur in the gut of many Pupipara (Diptera) which are blood sucking ectoparasites of many domestic animals (203). The drug store beetle, *Sitodrepa panicea*, has symbiotic yeasts in the blind sac of the midgut. They apparently furnish two thermostable substances which also occur in brewer's yeast (205). Coleoptera are well supplied with bacterial symbionts which lie in mycetomes of many different shapes, usually connected with the digestive tract (363). In the wood-boring family, *Bostrichidae*, bacteria inhabit mycetomes which always are isolated from the alimentary tract. They are transmitted to the egg by the process of mingling with the sperm and entering through the micropile at the time of fertilization (249). The function of such symbionts is quite unknown. However, the elaborate proc-

esses for transmission to the offspring that have been found in many widely different insect orders indicate true symbiosis, and probably varied physiological functions, but this point is strongly disputed (249). The subject of plant and animal symbiosis, with particular emphasis on the relations of insects is reviewed up to 1930 by Buchner (49).

Wing production. A feature of insect development which has attracted attention for many years is the production or non-production of wings by individuals of a given species. Aphis afford particularly good examples of such variations. Among the many conditions which have been considered as possible influencing factors are light, temperature, crowding, starvation and chemical composition of the host plant. In the case of the mealy plum aphid, *Hyolopterus pruni*, it has been suggested that lack of an adequate supply of food for the mother leads to winged offspring and that the above factors all exert an effect through influence upon nutrition (362). The order of decreasing importance as regards wing production of aphis has been stated to be light, temperature and condition of the parents as regards wings (354). The hypothesis that insufficient oxidation of the body tissues leads to the apterous condition, has been shown to be false in the case of several species of butterflies (105). It seems possible that wing production may be considered from the viewpoint of relative rates of growth but apparently no attempts have been made to do so.

Reproduction. The production of eggs and sperm by adult insects is dependent upon a supply of the necessary materials from which they are formed. In some insects these materials are sufficiently provided by feeding during the immature period but the majority require additional food after becoming adults. The Mediterranean flour moth, *Ephestia kuhniella* (Lepidoptera), requires neither food nor water, but the fig moth, *E. cautella* and the chocolate moth, *E. electella*, cannot produce their normal number of eggs without water. Sugar or albumen solutions do not increase egg production more than water alone (292). On the contrary houseflies and blowflies require protein or peptone for maturation of ova (204), (243). In males of the blowfly, *Phormia regina*, sperms are produced from one to three days after emergence and continue throughout the life of the fly. Protein is not needed and in fact starvation inhibits sperm formation only when death ensues (72). The bedbug, *Cimex lectularius*, is able to produce eggs without feeding in the adult stage. Such starved females lay 20 to 40 eggs over a period of four days (195).

It is characteristic of many mosquitoes not to complete the formation

of eggs until after ingesting a blood meal. But among *Anopheles maculipennis*, those females which emerge late in the fall use a blood meal to form the fat body and overwinter with immature ovaries (352), (115), (221). After hibernation, a blood meal in the spring is followed by rapid egg development unless they are again chilled in which case the fat body is replenished (149). The females of *Culex pipiens* are able to lay one batch of eggs without a blood meal only if they have had abundant food as larvae. And it has been claimed that the eggs are formed, not from the fat body, but from histolyzed muscle masses of the abdomen. Similarly in hibernating adults the fat body is formed from the same material (330). Adult females of this species, even though reared from undernourished larvae and unable to form eggs, are unwilling to seek blood until after mating (413). Iron is found in the vitellus of mosquito eggs and the necessity for iron in the larval diet, if the females are to form eggs without a blood meal, is satisfied by rearing the larvae on cooked lentils (39). Both sexes of adult fleshflies, *Lucilia sericata*, change in weight depending upon their diet. On no food or water only they lose steadily. On sugar and water both gain rapidly during the first day when the fat body grows. If given meat, the females have another period of rapid gain on the fourth to sixth days when the ovaries develop (98).

Nature of digestive tract. Insects feed upon a very great variety of organic substances and it is to be expected that their digestive organs will be similarly varied in construction and function. Aside from the mouth parts, three regions may usually be distinguished, foregut, midgut and hindgut. The foregut and hindgut, being of ectodermal origin, are always lined by a chitinous membrane which is usually shed at each molt. At least eight characteristic types of insect digestive tract have been suggested and are schematically illustrated in Wigglesworth's textbook, page 43 (427b). The rear portion of the foregut is often expanded into a crop for storage of food. Among the higher dipterons a variation of this condition is the development of diverticula or blind sacs connected only with the foregut by narrow ducts. Blood is normally taken into the midgut directly, but water, plant juices and other such liquids may pass into the ventral diverticulum along with any air accidentally taken into the feeding tube (240), (255). The diverticulum is usually not a digestive organ except as enzymes from the saliva are carried into it and anything which passes into it must go to the midgut before it is assimilated. A peculiarity of many insect larvae which are internal parasites, or which live in prepared cells stored with food

is that the mid and hindgut are not joined until just before growth is complete, e.g., *Cryptochaetum iceryae*, a dipterous parasite of certain coccids (387).

A structure characteristic of many widely varying species of insects is the peritrophic membrane. This is a chitinous tube extending through most, or all of the midgut and enclosing the ingested food. The function of this structure has received considerable attention, but it is generally agreed that it protects the cells of the midgut from injury by hard or sharp particles of food and thus acts in the same way as the mucus which is secreted in the digestive tract of mammals. In the lower orders of insects all cells of the midgut secrete a material which forms a sheet or series of sheets and it is strongly urged by some workers that this is the way in which all peritrophic membranes are formed (78). Probably midgut cells are concerned in all cases in which a membrane of several thicknesses is produced. The other viewpoint is that certain cells at the junction of the fore and midgut secrete material which is pressed into a tube by the infolded proventriculus (417). In larval Lepidoptera no anatomical structure for forming the tube is present but incoming food performs the same function (156). An embryological study of the honey bee and of certain fungus gnats (Mycetophilidae) indicates that the cells which give rise to the peritrophic membrane are derived from the stomodeum and hence are of ectodermal origin (54). This is consistent with the idea that chitin is exclusively a product of the ectoderm.

Whether the peritrophic membrane is formed continuously has not received much attention but in the tsetse fly formation is stimulated by a blood meal. The trypanosome organisms, *T. congolense*, *T. grayi*, and *T. brucei*, develop chiefly in the space between the peritrophic membrane and gut wall and their distribution at various periods after a meal of infective blood is only explainable if they are unable to pass through the membrane (169), (442). But solutes and colloidal material varying in size up to large aggregates such as Congo red pass through it readily (275), (78). The petroleum fly larva *Psilopa petrolei*, contains crude oil throughout the length of its digestive tract but the peritrophic membrane is not penetrated (386).

Nature of secretions of digestive tract. Intermittent formation of a cylindrical bag about the food mass has been described in the termites. The formation of this peritrophic membrane apparently is the result of a special secretion from the midgut epithelium and follows a period of intense production of secretory droplets which make up the digestive fluid (63a).

The pH of the parts of the digestive tract varies widely in different insects but extreme values are seldom reached. The saliva of several species of grasshoppers and locusts varies in pH from 4.9 to 5.8 (219); while that of the cockroach, and of the tsetse fly, is neutral (418). Numerous glands opening around the mouth parts of the honeybee have secretions that are slightly acidic (214). On the other hand the saliva of the sugar beet leafhopper *Eutettix tenellus* is decidedly basic (104). In the foregut no secretions are produced and the pH depends upon the food and upon liquids from the midgut which may move forward into the foregut in some insects.

Attempts have been made to show that the contents of the midgut are alkaline in phytophagous insects and acidic in carnivorous ones, but exceptions are very numerous and bacterial action may alter conditions. It is particularly interesting that the pH inside the peritrophic membrane of several species of culicine mosquito larvae is 6.2–8.2 whereas outside of the membrane it is 8.6 to 9.8 (319). This seems peculiar in view of the great penetrability of the peritrophic membrane but bacterial action may explain the difference. In adult female mosquitoes, *Culex pipiens* and *Anopheles maculipennis*, the pH of the gut averages 6.9 while that of the bedbug, *Cimex lectularius* is 6.2 (304). The sudden decline in incidence of primary malaria prior to the hibernation period of *Anopheles* sp. may be due to the fact that at that time ingested blood is sent into the diverticulum whose condition is alkaline enough to kill the malaria organism, for highly infective avian blood fed to *C. pipiens* in such a manner that it entered the diverticulum, failed to induce infection in the mosquito (241). A surprisingly low pH, 3.0–3.5, has been found in the middle portion of the midgut of fleshfly larvae (171). This acidity has been attributed to the secretion of phosphoric acid and may partly account for the sterilizing of wounds by the larvae (366). This acidic region is succeeded by a basic one, pH 8, in the latter portion of the midgut. A similar region of strong acidity, pH 3, has been found at the juncture of mid and hindgut of the damp wood termite, *Zootermopsis angusticollis*. This region is short and is preceded by pH 5.2 in the midgut and succeeded by pH 6.8 in the hindgut (315). The midguts of such different insects as the cockroach, the tsetse fly (417) and the honeybee have practically the same pH, 6.2–6.4. In the latter insect this pH is not concerned with the food, for in bees fed only distilled water for several hours, the pH is the same as those fed sugar water or honey (182). Conditions within the hindgut are complicated by the accumulated products of bacterial action and by excretory material from the Malpighian tubes which

enter at the beginning of the hindgut. Hence any general statement as to pH is impossible. In the case of the larvae of the fleshfly, *Lucilia sericata*, the contents of the hindgut and the excreta are strongly alkaline on account of the presence of ammonia (171). This occurs also under sterile conditions which signifies that ammonia is one of the principal end products of protein metabolism in this insect (270). On contaminated food, however, ammonia is produced sooner (172).

The buffering power of digestive fluids of insects has received very little attention. The contents of the gut of Japanese beetle larvae are somewhat basic and the pH is not affected by variations of the soil in which they live (373). The secretions of the ventriculus (midgut) of the worker honey bee are buffered by two systems, one composed of mono- and dihydrogen phosphate at concentration 0.046 M with maximum buffering strength at pH 6.8 and the other probably composed of an organic acid and its salt, at concentration 0.163 M and with maximum buffering power at pH 4.1 (182).

Digestive enzymes. Uncertainty has long prevailed as to the part played by microörganisms in the production of the enzymes found in the digestive tracts of insects. It has been shown that in at least two species of termites, symbiotic flagellates are responsible for the production of cellulase (276), (396). A primitive, wingless roach, *Cryptocercus punctulatus*, harbors in its hindgut some species of protozoans which have previously been found only in termites (64). From this circumstance, conclusions have been drawn regarding the common ancestry of termites and roaches and the period at which they diverged. These protozoans form the enzyme cellulase and the roach can live on its natural food, wood, only when they are present. Cellulose is changed to cellobiose and then to dextrose in the termite's hindgut but no absorption occurs there and the digestate must be forced back into the extra-peritrophic region of the midgut. Another wood-feeding roach, *Panesthia javanica*, has cellulose-digesting bacteria instead of protozoa. But bacteria probably have little to do with the digestion of cellulose by termites (82). The wax-moth larva is able to digest beeswax and the wax produced by tubercle bacilli, probably with the aid of esterases from the symbiotic bacteria of the gut (83).

Cellulase has been found in a number of wood-feeding insects which do not harbor microörganisms (324) and in certain cases it has been shown that *Cerambycid* beetle larvae select wood in accordance with the ability of their enzymes to digest cellulose (251). Wood-feeding insects have been divided into three classes: those which produce cellulase

and can utilize cellulose; those which have none and are restricted to wood containing much starch and sugar; and those such as termites which harbor symbionts that can digest cellulose (248). In the latter case the microörganisms probably are an important part of the host's food (250), (252), (414).

The enzymes produced by fleshfly larvae have received attention in connection with the rearing of these insects on sterile food. Under these conditions only amylase is found in the saliva and tryptase, peptidase and lipase in the midgut (109). The tryptase has an optimum pH at 7.5 and is nearly inactive at pH 4 which indicates that most of the protein breakdown occurs in the rear portion of the midgut and not in the strongly acid central portion. An enzyme which digests collagen in basic solution is also produced by the cells of the midgut (170). These enzymes in part pass out in the excreta and cause extra-intestinal digestion of the larval food, especially after enough ammonia has been formed to make the food alkaline.

It may be said that, in general, the digestive enzymes of insects are adapted to handle their ordinary food, and those which could have no function are not formed. Thus, the proteolytic enzymes which predominate in fleshfly larvae are largely absent in the adults which subsist mainly upon carbohydrates and are well supplied with invertase and similar enzymes. The honey bee is an example of an insect whose diet is particularly rich in carbohydrates. The secretions of the salivary glands and of the midgut contain a very active invertase whose quantity varies with the bee's age in accordance with the duties performed in caring for the young and preparing honey at different periods of life (210). Typical blood-sucking insects such as the tsetse fly are well supplied with proteinases while those whose diet is more generalized have enzymes for digestion of all classes of foods (417), (166). Very unusual diets are accompanied by unusual enzymes as illustrated by the clothes moth larva which produces a keratinase for digestion of the keratin in wool (394). The locust, *Calliptamus italicus*, whose normal diet is entirely plant material, has amylase, cellulase, inulinase, saccharase and maltase in the salivary glands and the midgut (374).

The different regions of the digestive tract vary greatly in their secretory and absorptive functions. The midgut is usually the chief site of both these processes, but the varying length of the hindgut in relation to food habits shows that it plays an active part in digestion. No special secretory glands occur in the Japanese beetle but all the epithelial cells secrete digestive fluids (372) and this is probably the usual situa-

tion. In the cockroach the epithelial cells which line the anterior part of the midgut are absorptive when young and become secretory when older. Mitochondria give rise to secretory granules which crowd into the lumen end of the cells during the secretory stage. This is characterized by bursting of the cell and discharge of the granules into the lumen. This process may occur several times before the cell finally degenerates and is replaced by young, absorptive cells (130). An essentially similar process occurs in the grasshopper (433) and in cerambycid beetle larvae (92). Definite nests of cells of varying age have been described in the midgut of the termite, *Zootermopsis nevadensis*. The older cells are secretory and are continuously replaced by younger ones (63a).

The diverticulum of mosquitoes is not an absorptive organ, for a formaldehyde solution is non-toxic as long as it enters that organ but causes instant death when sent into the midgut (240). The anterior portion of the midgut of *Lucilia sericata* (fleshfly) larvae by means of peristaltic movements pushes food into the middle segment where water, whose liberation from the food is assisted by the pressure, is rapidly absorbed. The principal absorption of foodstuff occurs in the posterior segment of the midgut (171). Absorption occurs chiefly in the same part of the midgut of the cockroach (130). In termites the midgut and hindgut are absorptive (276). In the honeybee there is a cycle, for phosphate is secreted in the midgut and absorbed in the hindgut so that very little occurs in the rectum or is lost in the feces (182).

Storage and utilization of foodstuffs. It is unfortunately true that very little is known about the processes of metabolism in insects or where they take place. The presence in the digestive tract of enzymes capable of splitting carbohydrates, fats and proteins into smaller units makes it probable that it is these products which penetrate the wall of the digestive tract. Carbohydrates vary greatly in their nutritive value, only hexose sugars and disaccharids being utilized by a number of lepidopterous larvae. Starch is not assimilated (47). The honey bee is unable to make use of pentoses (407). Glycogen occurs in adipose cells of the fat body of *Culex pipiens* but not in the intestinal cells during digestion. Hence it is probable that glycogenesis occurs in the fat cells (38).

Many insects have been found to have the ability to synthesize fats from carbohydrates. The adult anopheline mosquito (149) and the adult leafhopper, *Eutettix tenellus* (121), can form fat when fed only on

sugar water. The larvae of the fleshfly *Lucilia sericata*, is able to form fat from protein. Unpublished work in this laboratory has shown that the nature of the diet affects the properties of the larval fat. The percentage of fat varies enormously in various insects and is dependent upon the stage in the life cycle as well as the condition of nutrition. Thus *Lucilia* larvae contain about 20 per cent fatty material on the basis of dry weight, and the adults very little. On the other hand, nearly 50 per cent of the dry weight of hibernating *Culex pipiens* females has been found to be fat (59).

Excretion. Many modes of excretion have been suggested for disposal of the waste products formed by insects. But, although various cells within the body and certain parts of the outer covering may have a part in the process of elimination, the system of fine tubes known as the Malpighian tubes is undoubtedly the chief excretory organ. These tubes vary in number from two to more than one hundred. They empty into the digestive tract at the juncture of mid- and hindgut and important changes in the urine may occur before it is voided. The many variations in structure and arrangements of insect excretory systems fall into at least six classes (427c), which differ mainly in kinds of cells in the different portions of the tubes, association of their distal ends with the midgut, and substances found within the lumen. In the simplest case the tubes lie free in the hemolymph, consist of only one kind of cell and excrete a clear liquid. One variation is illustrated in the bloodsucking bug, *Rhodnius*, which has four tubes in which two regions of different kinds of cells occur, and solid excrement is present in the lower region (419).

Another important difference in some insects is the close application of the closed distal ends of the tubes to a region of the hindgut below the point at which the proximal ends empty into it. This is the arrangement in many beetles, e.g., Tenebrionidae (302), Dermestidae (254), and in lepidopterous larvae. This arrangement seems always to be found in insects whose excrement, both from the gut and from the Malpighian tubes, is finally expelled in a very dry condition. The value of this arrangement is obvious for insects which feed on very dry substances, for conservation of water is one of their main problems, but its value is not clear in the case of many caterpillars which eat leaves very high in moisture. A particularly careful study has been made of the relation of the Malpighian tubes to the hindgut in the case of the meal worm, *Tenebrio molitor*. The lower ends of the six tubes are closely bound, within a common sheath, about the hindgut just before

the rectum. Their function in this region is the removal of diffusible toxic and waste substances which are carried through the gut wall by water as it is extracted from the feces. Such materials are transported down the tubes while much of the water passes into the hemolymph (302), (303).

The substances excreted by insects are doubtless as varied as may be expected from their diverse feeding habits, but little information is available except with regard to salts and certain end products of protein metabolism. The larvae of the soldier fly (Stratiomyidae) and the moth fly (Psychodidae) normally live in water very rich in dissolved calcium salts and during each instar solid calcium carbonate accumulates in the Malpighian tubes. During each molt these deposits dissolve and reappear as incrustations on the cuticle of the stratiomyid larvae. The psychodid larvae are encrusted in a different way and the external deposit is not derived from the lime salt in the Malpighian tubes (218). Larvae which live in acidic water, e.g., chironomid larvae. do not have deposits of calcium carbonate in their Malpighian tubes.

The urine of the bug, *Rhodnius*, is a clear liquid for several hours after a blood meal, during which time most of the water from the ingested blood is eliminated along with most of the sodium and potassium chlorides and carbonates (419). A little sulfate and a little calcium also occur but no iron or magnesium. The initial pH is about 8 and the liquid is approximately isotonic with the ingested blood. After the first day the urine decreases in quantity, gradually becomes slightly acid, pH 6, and is progressively concentrated until it becomes a semi-solid. In this condition, which persists for about a month after a full meal, the main component of the excretion is uric acid, along with a little urea and alkali urates. The distal two-thirds of the Malpighian tubes always contain clear liquid, but in the proximal portion the contents are crystals of uric acid. The suggested mechanism is that sodium and potassium urate are excreted into the lumen of the upper portion of the tubes and that in the lower portion the metals are returned to the hemolymph, probably as carbonates, and solid uric acid is precipitated. This is consistent with the experimentally determined fact that the pH is 7.2 in the upper region and 6.6 in the lower. It is also of interest that no reducing sugar or ammonia occur in the urine of *Rhodnius*.

The excretion of flesh eating larvae of the fly, *Lucilia sericata*, is in sharp contrast to that of *Rhodnius*, for ammonia is one of, if not the chief end product of protein metabolism (170), (171), (270). It has not been shown that ammonium salts are excreted by the Malpighian

tubes but it seems probable that this is the case. As the butterfly, *Deilephila euphorbiae*, leaves the pupal case it excretes a semisolid mass consisting mostly of uncombined uric acid but nearly ten per cent of the total nitrogen is present as ammonium salts. The pH is 5.8–6.3 (152). It has been found that in silkworms uric acid is derived not only from metabolism of histidine and arginine but from amino acids in general (3) and these insects thus resemble birds and reptiles.

The Malpighian system is not always able to discharge uric acid as fast as it is formed for this substance is but slightly soluble and if produced in large quantities, as during the histolytic stage of ecdysis or metamorphosis, it will be precipitated where formed. This is the case with the bug *Rhodnius* which has uric acid and a red pigment, which is also excretory, in the epithelial cells only during molting (424). Similarly, a red pigment is excreted by the Malpighian tubes of lepidopterous larvae just before pupation and is also deposited in the cuticle (268).

Excretory pigments. The waste products of insect metabolism are often colored and their deposition in various tissues of the body accounts for some of the coloring that is so widespread in insects. A great body of data has been published over a period of many years relating to color due to pigments as distinguished from that due to structure of the cuticle (79), (189), and the chemistry of insect pigments is established fairly well in some cases. But the relation of pigmentation to physiology is but slightly understood. Probably the simplest situation is that in which pigments of the food are deposited unchanged in the cuticle. Many insect colors varying from yellow to red are due to ingested carotene or allied compounds often classed under the general name of carotenoids. In some cases chlorphyll from plants is deposited in the cuticle of larvae which feed upon them. On the other hand no chlorophyll could be found in the bodies (digestive tract removed) of green specimens of the locusts, *Locusta migratoria migratoriodes* or *Lucustana pardinala*, and the color was not dependent on chlorophyll in the food (101). Caterpillars break off and digest the unsaturated alcohol phytol from chlorophyll but do not carry the degradation as far as occurs in ruminants (106). The green coloring matter in the hemolymph of several species of lepidopterous larvae is not from chlorophyll but is an oxidation product of proteins (268). The white pigment, leucopterin, which occurs in the wings of many butterflies is considerably different from uric acid but gives a modified murexide test (415).

The complex pigment melanin is widely distributed in insects. It is formed from several procursors by the aid of oxidative enzymes which may be specific for each substance that is oxidized. Tyrosin was the first procursor to be identified but "dopa" (dioxyphenyalanine) occurs in the elytra of *Melolontha* beetles and dioxyphenylacetic acid in the mealworm, *Tenebrio molitor* (343), (344). Encapsulation of internal parasites is accompanied by production of melanin (90). The processes by which the procursors of melanin are formed are not known but it is possible that the production of this pigment is a method for disposing of phenolic compounds since they seem to be required for its elaboration.

The walking stick insect, *Dixippus morosus*, changes color in response to a variety of stimuli such as temperature, mechanical pressure, and colored surroundings. Sectioning of the eye stalk eliminates response to visual stimuli and removal of the front lobe of the tritocerebrum stops all color change. Hence it is concluded that a nerve center of the brain causes the formation or liberation of a hormone which governs the movement of pigment in the outer covering of the body (12). Crowding of the locust, *Locusta migratoria migratoriodes*, is followed by the development of a dark color (101). It has been suggested that the great activity which occurs under crowded conditions gives rise to a black colored product of metabolism, probably a waste product, in such quantity that it cannot be handled by the Malpighian tubes and is deposited in the cuticle. This substance, called locustine, may be included in the eggs and causes the first nymphal stage to be dark colored.

Water balance. Water constitutes a large proportion of the food of most insects, and ordinarily the excess is excreted without difficulty. But under conditions of high humidity the mealworm, *Tenebrio molitor*, produces more metabolic water even from its dry food than can be eliminated readily (56). But, ordinarily, the difficulty is in conserving enough water. It has been found that, except for extreme conditions, the rate of loss of water from insects is proportional to the saturation deficiency of the atmosphere, i.e., to the amount of water by weight which must be added to a unit volume of air to bring it to 100 per cent relative humidity. This rule holds for the cockroach which prefers successively lower temperatures as it loses water (132). The same is true for the clothes moth, *Tineola biselliella* (263) and the starved bedbug, *Cimex lectularius* (260a), which both contain higher proportions of solid matter when kept in dry air. On the other hand the mealworm

maintains a constant percentage of water in its body when starved for a month at relative humidities of 0 to 60 per cent (56). This insect and hibernating nymphs of the grasshopper, *Chortophaga viridifasciata*, are able to absorb moisture from air which is considerably below saturation (59), (261). The termite, *Zootermopsis angusticollis*, however, cannot take up an appreciable amount of water even from a saturated atmosphere (69). The tsetse fly is able to maintain a constant proportion of water in the body over the range of 11 to 88 per cent relative humidity by burning more fat when placed in dry air (62). Overwintering pupae of the butterfly, *Deilephila euphorbiae*, are in equilibrium at 20°C. with air of 99 per cent relative humidity. They lose water at an increasing rate until the relative humidity falls to 80 per cent, but during further decrease to 48 per cent the rate of loss is practically constant (154). It has been suggested that in very active insects most of the water loss is by way of the spiracles and hence dependent upon the rate of respiration. This is governed by many conditions other than saturation deficiency, so close correlation should not be expected (263). This is probably the case with *Aëdes aegypti* mosquitoes (228) and with rat fleas (225) whose survival without food is dependent upon humidity, but is not directly related to the saturation deficiency.

Insects which live in the water have no need to conserve this substance. In the mosquito larva (*Aëdes aegypti*) special blood gills located in the anal region are the principal site of water absorption or loss. This is governed by osmotic pressure so that the larvae shrink in hypertonic solutions and swell in hypotonic ones (421), (422). Adaptation can be made to sodium chloride solutions up to 1.1 per cent (423). Further consideration of these structures will be given in the discussion of respiration.

Limiting and fatal conditions. The extreme conditions of temperature and humidity under which an insect can live often are related to its ability to conserve water. A large proportion of the earlier attempts to find the upper fatal temperature did not take account of the effect of humidity nor did they clearly distinguish between short and prolonged exposures. The data up to 1930 are summarized by Uvarov (402b). The blood-sucking bug, *Rhodnius*, is very resistant to drying and survives a twenty-four hour exposure to 40°C. in air dried by concentrated sulfuric acid (57). But during a month's exposure to such dry air at only 23°C. the body fluids of the fasting insect become too concentrated for life to persist (58). In twenty-four hour tests, newly hatched human lice and adult mosquitoes are able to survive tempera-

tures five degrees higher (37–38°C.) when in moist air than they can in dry air (32–33°C.). The upper limit is not affected by humidity when the test is for only one hour (264). These observations are all consistent with the idea that the two insects can resist desiccation effectively for only a short time. When minimum summer temperature is above 24°C. adult *Anopheles maculipennis* do not live long enough for the malaria organism, *Plasmodium vivax*, to reach the salivary glands and consequently there is a decline in the incidence of malaria (44), (115). Similar observations have been made concerning *Anopheles stephensi* (367). A midsummer decrease in number of adult *Anopheles maculipennis* occurs in Spain (55) and in California (115) and in the latter region has been shown to be coincident with elevated temperature and low humidity. Laboratory experiments show that a relative humidity of 55 per cent insures the normal life span of this mosquito at 18°C. while no amount of atmospheric humidity will protect it at 23.5°C. Loss of body water even when the humidity is high causes the females to seek a blood meal to renew the water supply (228). A quite different situation prevails in the case of the tsetse fly (62) and *Leptinotarsa* sp. (Chrysomelid) beetle (61) which are more resistant to high temperature when they are somewhat desiccated. A generalization, based upon experiments with several kinds of insects, has been stated to the effect that death that occurs below 36°C. is on account of desiccation, while above 40°C. it is caused directly by temperature (260). Apparently this rule does not hold for the mosquito, *Anopheles maculipennis*, mentioned above. An attempt to show that death of adult grain beetles at high temperature is due to inactivation of the enzyme catalase gave negative results (301a).

As the temperature rises, all insects become more active, but the temperature of maximum activity depends upon the previous conditions. Thus, if groups of blowflies are each kept at a constant temperature, it will be found that maximum activity occurs in the middle of the range in which activity is possible, but if the flies are slowly heated, it occurs near the upper temperature limit (289). In many insects this upper activity limit is characterized by a heat rigor or paralysis from which recovery is possible if the condition does not last long. In the case of the common housefly (290) heat rigor occurs at 45.4°C. and with the stablefly, *Stomoxys calcitrans*, at 42.6°C. (291). With neither insect does humidity have an appreciable effect. The wasp, *Vespa orientalis*, becomes normally active at 23°C., reaches greatest activity at 35.4°, heat rigor at 41.8°, and death at 44°C. (25). It

may be said, in short, that temperature affects any metabolic process in the same way previously discussed in connection with growth.

The effects of low temperature upon insects are of great importance in connection with their survival during winter and with the protection of materials on which they feed. The importance of humidity and length of exposure should be noted in connection with low temperature as well as with high. A summary of the data up to 1930 is given by Uvarov (402a). The larvae of the yellow fever mosquito, *Aëdes aegypti*, will revive after being kept at 2°C. for as long as ten hours, but after eleven hours, none survive (21). Freshly laid eggs were killed in a few days at 10°C., but if exposed when nearly ready to hatch, they were not affected adversely in eight weeks though hatching was delayed (76). The larvae of Aëdes mosquitoes survive freezing for over eight hours, but not for twenty-four hours (314). It is stated that the tropical bedbug, *Cimex hemiptera*, which cannot survive a temperature of 9°C. for three months, does not spread into Japan because the houses are not heated in the winter (294). An example of protection of foodstuff on account of the ready susceptibility of an insect pest to destruction at low temperature is afforded by the confused flour beetle, *Tribolium confusum*. All stages of this insect are killed at 7°C. in twenty-five days, but at −6°C. only twenty-four hours are needed (283). An interesting discussion of the effects of climatic conditions upon the population of insects, especially those of medical importance, has been given by Buxton (61).

Internal temperature. Many social insects control the temperature of their living quarters by coöperative actions (164) such as aiding ventilation by moving their wings during hot weather. Meteorological data are often misleading with respect to the actual conditions under which insects live in a given community, for they may inhabit microclimes very different from the general conditions. Thus the adults of several species of mosquitoes have been found to survive periods of high temperature by staying in wet grass or near small pools which are cooled by evaporation of water (262). However, the depression of temperature on account of evaporation is a function of the humidity and conditions of high temperature and high humidity may be fatal to water inhabiting insects such as mosquito larvae, because their environment remains cooler when the humidity is lower (226). The foregoing observations illustrate the care that must be used in correlating insect survival or abundance with the ordinary weather data.

Radiation may play an important part in determining the body tem-

perature of insects. Thus it has been found with hoppers of the desert locust, *Schistocerca gregaria*, that they are warmer during the day than the surrounding air or the surface of the ground (22). Moreover their body temperature drops very rapidly during periods when the sun is temporarily obscured. Somewhat similar results have been noted with the grasshopper, *Oedipoda coerulescens* (114). Many insects either increase or lower their body temperature to the optimum by altering the angle at which the body is held to the sun (217), and many attempts have been made to interpret insect coloration in terms of requirements for absorption or reflection of radiation (337), (308). Large insects are able to maintain their body temperature distinctly below that of the surrounding air if it is relatively dry and neither the temperature nor length of exposure is extreme. Thus, the hawkmoth was cooler by 2.5°C. than surrounding dry air at 47.6°C. but was unable to maintain a difference at 51.5°C. and in moist air its body temperature exceeded that of the surroundings (59). Electromagnetic waves of high intensity raise the internal temperature of insects, e.g., larvae of the Japanese beetle were heated to 42° and killed at once in a field of 4000 volts and 3 million cycles (147).

Hibernation and diapause. When placed in an unfavorable environment many insects enter into a dormant state in which all physiological processes proceed at a very slow rate and some, such as growth, apparently cease altogether. Such a condition may prevail at any stage of the life cycle. Two situations are to be distinguished; first, the resting condition is brought on by unfavorable temperature, humidity, etc., and promptly ceases when the environment becomes favorable and, secondly, the condition comes on at a definite period in the life cycle and neither its initiation nor ending is dependent upon the environment. Various terms have been used to designate the two conditions but hibernation and diapause seem to be more widely used than any other expressions. With some insects it is difficult to classify their dormancy definitely as one or the other for intermediate conditions exist, e.g., the sandfly, *Phlebotomus papatashi* overwinters in Palestine in the larval condition, but there are both active and resting larvae present together during the summer (381). Different intensities of diapause occur among the larvae of the mosquito, *Anopheles plumbeus*, so that they develop at very different rates during the winter (332). Adult *Anopheles maculipennis* continue to feed during the winter in England, but the time for digestion of a meal of blood increases greatly with falling temperature, being two days at 26.5°C., five days at 15°C. and

about thirty days at 4.5°C. (355). Among other peculiarities are variation in the larval instar at which diapause of the Japanese beetle occurs (230) and the prolonged diapause which a small percentage of the eggs of the yellow fever mosquito undergo (133). Sudden reduction in temperature does not bring on dormancy of the polyphemus moth, *Telea polyphemus*, but progressive reduction is highly effective and the temperature level through which reduction is made is not of much significance (77). During diapause, larvae of mud-dauber wasps are much more resistant to x-radiation than during active development. If developing larvae are irradiated and then immediately chilled to the normal temperature of diapause, the harmful effects of irradiation are greatly lessened (36).

A number of miscellaneous determinations have been made with regard to body constituents during hibernation or diapause. Cold resistance seems always to be associated with a reduction in free, i.e., solvent, water in the insect body. In some cases the total water is reduced. Hibernating caterpillars of the brown tail moth, *Euproctis chrysorrhoea*, have larger percentage of dry weight, less freezable water and higher fat content than do those fed for a few days before being analyzed (338). Larvae of the rice borer reduce the water content of the body and freezing point of the blood as winter continues (349). Fatty acids in the eggs of grasshoppers which overwinter in that stage have a lower melting point (359) and a higher iodine number than do those in eggs which occur only during the summer (360). The catalase content of army cutworm moths is directly correlated with their degree of activity during the summer and declines sharply during their summer dormant period or estivation (301).

Diapause may be broken in a variety of ways. Dormant larvae of the mosquitoes, *Anopheles bifurcatus* and *A. maculipennis*, can be made active by adding a little potassium permanganate or other oxidizing agent to the water in which they are spending the winter (341). The diapause of pupae of the walnut husk fly, *Rhagoletis completa* may be broken by a number of chemicals such as ethylene chlorohydrin and potassium thiocyanate (43). Parasites often hasten the development of the host. Thus gall-forming Diptera (Chloropidae) normally overwinter as larvae, but when parasitized by a Brachonid, they pupate in the fall (405). But when the larvae of *Sciara pullula* (Diptera) are parasitized by a nematode their pupation is delayed (196).

The various hypotheses that have been advanced from time to time to explain the phenomenona of diapause have usually included the idea

of interruption of the production of a hormone which promoted whatever feature of metabolism was under consideration. The opposite theory, that a factor retards development and can be destroyed by cold, has been elaborated for the case of grasshopper eggs (31). If this idea has general application it would follow that chemicals, mechanical shock, etc., also can destroy the retarding substance.

The increased resistance of insects to extremes of cold which accompanies the diapause condition is of great importance in connection with their distribution. The northern limit of spread of the Colorado potato beetle had been attributed to the fact that its lethal limit is between -5 and -12°C. (246). The western pine beetle, *Dendroctonus brevicomis*, in districts in which it regularly goes into a dormant condition is much more resistant to extreme cold than it is in warmer climates (271). A very good review of the whole subject has been given by Imms (190) and further consideration is scarcely appropriate here since it is really a branch of ecology rather than of physiology.

Respiration. Knowledge of the respiration of insects has been advanced in comparatively few directions during recent years, but some important additions have been made. An excellent summary up to 1930 has been published by Wigglesworth (420) together with a comprehensive bibliography of older publications.

Respiratory movements. The respiratory movements of insects are usually limited to two kinds, rhythmical change in size and shape of part of the body, and opening and closing of the valves which are located where the tracheae reach the outer surface of the body. Expiration is active and is brought about usually by contraction of the abdominal muscles. Inspiration may be either active or passive, i.e., due to elasticity of the body wall. In certain water beetles, the metathorax performs the respiratory movements and the abdomen plays only a passive part (45). The nervous impulses responsible for these movements apparently come from the nervous ganglia, for in the case of the diving beetle (*Dytiscus*) and the dragonfly (*Aeschna*) it has been shown that the isolated ganglia show alternations in potential which correspond in frequency with the respiratory movements of the insects (2). While resting insects often make no visible movements in connection with respiration, some may start vigorous movements in anticipation of flight (113). This appears to occur purely by nervous action but ordinarily chemical stimuli are the real controlling influences. Lack of oxygen or excess carbon dioxide are of most importance. The chief nervous centers are located in the thorax rather than in the head, since

decapitation interferes but little with respiratory movements except for the initial shock.

Ventilation. Alterations in the volume of the body obviously serve the purpose of forcing air into and out of the respiratory system. The trachea however are stiff-walled and can change their volume but little. But the large cavities, or air sacs, which are particularly abundant in very active insects, are easily compressed (131). The respiratory volume may amount to one-third to two-thirds of the total capacity in the few insects that have been investigated (420). It was debated for many years whether a directed flow of air takes place, i.e., air enters at one region of the body and is discharged from another. All doubt that this occurs in certain insects has been set aside by recent experiments. By fastening the grasshopper, *Chortophaga virifasciata*, in a partition between two chambers so that they were connected only through the respiratory system of the insect, it was found that air enters the thoracic spiracles and leaves by those of the abdomen. Volume per minute per gram of body weight averages 0.11 cc. at 23°C. and 0.23 cc. at 28°C. (236). The rate is increased by carbon dioxide, and momentarily by hydrogen cyanide or nicotine at low concentrations (237).

The direction of flow is similar in the migratory locust, *Schistocerca gregaria*, and it was shown that during flight of this insect and of the wasp, *Vespa orientalis*, the ventilation is accelerated (112), (113). Unpublished data obtained in this laboratory show that ventilation from front to rear also occurs in the cockroach, *Myctobara noctivoga*.

It is obvious that a directed flow of air through the tracheae requires coördination between the respiratory movements and the action of the spiracle valves. The greatest problem of most land insects is conservation of their water content and consequently the spiracle valves are kept closed as much as possible to reduce evaporation by way of the tracheal system from which most of the water loss occurs (263), (207), (208). A particularly important observation has been made on the behavior of the spiracles of scale insects. When exposed to sublethal concentrations of hydrogen cyanide, the spiracles close tightly and the resultant "protective stupefaction" makes the insect very resistant to cyanide fumigation (310). The precise nervous control of the spiracle valves is not determined, but it must reside in some part of the central nervous system at least while active ventilation of the tracheal system is taking place. Reversal of direction of flow through the body when the thorax is exposed to toxic gases is additional evidence for central control of the process (237). In the case of Vespa sp. (social wasp)

it has been claimed that three ganglia, the thoracic, the fifth abdominal, and the eighth abdominal are concerned in spiracle action and respiratory movement (113).

It should be emphasized that observations on respiratory movements of the abdomen tell little about directed flow of air through the body, for experiments in this laboratory have shown that in the presence of nicotine, spiracular action of the cockroach sometimes ceases but active respiratory movements continue. Of course, directed ventilation cannot occur then. In many insects, especially those with incomplete respiratory systems, forced movement of air in and out of the same spiracles is the chief method for renewing the air in the body, e.g., with mosquito larvae and pupae. Insects which live either in water or on land may respire differently in different environments, e.g., the larva of *Cybister* (Dytiscidae) inspires only through the last pair of spiracles and expires through all spiracles when in the water, but out of water the lateral spiracles are inspiratory also (279). A particularly interesting variation in respiration is afforded by a water bug, *Corixa* sp., which carries a bubble of air with it beneath the water. When the carbon dioxide concentration in the bubble rises to 0.04 atmosphere, reflex movements of the legs begin and a current of water is forced to sweep past the bubble and carry away the carbon dioxide as it diffuses out of the bubble (42). Oxygen deficiency is not concerned with this for when that occurs the insect rises to the surface and draws down another bubble.

Diffusion. Forced ventilation is often of great importance in renewing air in the larger air chambers and tubes, but in the very narrow tracheoles, which may reach into individual fibers or cells, diffusion is of predominant importance. Recent writers are nearly all in agreement that at no time is the air in the respiratory system put under pressure to force it into the tracheoles, though a contractile phase with spiracles closed during the expiratory phase has been described in the case of the migratory locust (112). The presence of liquid in the terminal portion of the tracheoles has been suspected by many workers. This further complicates the problem of adequate oxygen supply to the tissues and of increased supply during activity. An explanation bearing on these points has been offered as a result of observations on the larva of the yellow fever mosquito, *Aëdes aegypti* (416). When the insect is at rest the innermost portion of the tracheoles is filled with liquid. Lack of aeration causes absorption of the liquid and the air column advances farther into the tissues. Admission of air results in

slow return of the liquid to its original level. Injection of hypertonic solutions of salts into the body likewise is followed by extension of the air columns. Hence it is argued that during muscular activity, with consequent using up of available oxygen and formation of products of metabolism, the liquid is withdrawn from the tracheoles by osmosis and gaseous diffusion thereby is given an opportunity to extend into the active tissues. The validity of the experimental facts has been corroborated in the case of the Coccinelid beetle, *Epilachna chrysomelina* (380) and in several other terrestial insects (418). It is evident that a delicate balance exists between the forces of capillarity and of osmosis, both of which have been estimated on the basis of fragmentary data to be of the order of ten atmospheres. Also the lining of the tracheoles must be very different from that of the tracheae near the spiracles for water does not seek to enter the latter.

Before an insect leaves the egg its tracheal system is filled with liquid, which is apparently not part of the amniotic fluid. At least three situations exist with respect to the first appearance of air in the system (357). First, the surface of the larva dries while still in the egg and air comes through the shell and enters the tracheae as the liquid is absorbed, e.g., the mealworm, *Tenebrio molitor*; secondly, air enters the tracheae only after hatching, e.g., the bedbug, *Cimex lectularius*. And thirdly, the tracheal system fills with air while the larva is still surrounded with amniotic fluid, e.g., the fleshfly, *Lucilia sericata*. In each case the activity that accompanies hatching operates to absorb liquid from the tracheoles and aids the entrance of air into the system.

Function of the blood in respiration. The importance of tracheal ventilation and diffusion in the tracheoles has been emphasized partly because insect blood is not an efficient carrier of oxygen. The walls of of the tracheoles and of the large air sacs are freely permeable to gases, however, and hence the blood must contain an appreciable amount of both oxygen and carbon dioxide. This is the more certain in some insects, e.g., *Nepa* (water scorpion), which have special organs richly supplied with tracheae and freely washed by the blood (137). Even in the case of the larvae of *Chironomus* (blood worms) which contain hemoglobin in solution in the blood (342) the binding power for oxygen is not adapted to aid in respiration except under very low oxygen tensions (139).

It was suggested several years ago that the copper-containing respiratory pigment hemocyanin occurs in insect blood but this does not appear to have been verified and in the case of one species of grasshopper

the concentration of copper is too low to make possible the presence of any appreciable amount of hemocyanin (318).

Nymphs of certain dragon flies and caddis flies have been considered to have a respiratory pigment in the blood though its nature is not known (162). In many insects, especially Lepidoptera, there is a rapid circulation of blood in the wings and the blood channels there are very thin walled. It is also true that such insects may exercise very vigorously without showing any respiratory movements (306). But if the wings are smeared with oil or the circulation of blood is stopped by piercing the pulsating organs at the bases of the wings the butterflies are unable to fly for more than a short time and show evidence of exhaustion. Hence it is likely that the wings serve as aerating organs for the blood which plays an important rôle in respiration (307). Since carbon dioxide is much more soluble than oxygen in water, it is probable that the principal rôle of blood in insect respiration has to do with the elimination of the former gas.

Aquatic insects. Special adaptations of the respiratory system to aquatic life occur in many species of insects. Some species depend upon bubbles of air which are held to the body at various places, e.g., beneath the wings in which position they are renewed periodically by an ingenious procedure (295). But tracheal modifications also occur. The most striking of these is the "closed" system, in which there is no connection of the air passages with the outside. At first sight this would suggest that the tracheae have no respiratory function, but the distribution of tracheoles to the tissues is the same as in insects which have spiracles and another system of very fine, thin walled tubes occurs in the integument and connects with the main air tubes (420). In certain regions of the body wall, usually along the abdomen or within the rectum the surface is increased by evaginations called tracheal or rectal gills. Structures similar in shape but filled with blood and not particularly rich in tracheoles also occur in aquatic insects. They seem to have little to do with respiration (390) but are of importance in connection with water absorption (421). The importance of the tracheal gills varies greatly among different species and even within an order differences exist as with the Trichoptera, some of which have well-developed, functional tracheal gills whereas in others they are rudimentary (389). In mosquito larvae the open tracheal system is the chief respiratory organ, as might be expected, but carbon dioxide is given off over the whole body surface (422). Nymphs of the burrowing mayfly, *Hexaginia recurvata*, have prominent tracheal gills which

can be dispensed with during the winter but are necessary for completion of the life cycle during the following summer (278).

Parasitic insects. Internal parasitic life has led to modifications analogous to those occurring in aquatic life. The early larval stages are often atracheate and depend upon respiratory exchange with the blood of the host through their own integument (387). At a later stage the tracheal system develops, and connection is made with the outside air. This may be done by means of a funnel-like opening through the integument of the host as in the case of *Zenillia libatrix*, a tachinid parasite of larvae of the gypsy moth and other lepidopteran species (85) and in the last larval instar of *Cryptochaetum grandicorne*, a dipterous parasite of certain coccids (391). A somewhat more elaborate arrangement is encountered in the case of *Encyrtus infidus*, a hymenopterous parasite of another coccid. In this case the spiracular areas of the parasite become attached to a number of tracheae of the host, so that the two respiratory systems are connected in one or more places (75). Progressive degeneration of the respiratory system as the habit of endoparasitic life is developed is illustrated by three braconid parasites of fly larvae. There is a striking correlation between the degree of development of locomotory structures, of head framework and of tracheal system (96). Another feature of the dipterous parasites (*Cryptochaetum*) mentioned above is the long caudal filament or tail which is supplied with both tracheae and blood and acts like a tracheal gill (387), (391). On the other hand it has been claimed that the long tail of certain Ichneumonid larvae is of no physiological importance, e.g., *Eulimneria crassifemur* which parasitizes the larvae of the European corn borer (384).

Gaseous exchange. The energy metabolism of insects varies enormously, but other conditions being the same, it may be roughly correlated with their relative intensity of activity. Thus, among the larger orders, it is highest in Hymenoptera, then Diptera, Orthoptera and Coleoptera (163). The great variation in energy output of resting and active honeybees has been variously stated as a three-fold increase when the bee is merely walking about (100) to a thirteen hundred-fold increase during flight (211). The gaseous exchange increases with temperature and in general follows the same behavior as was discussed previously in connection with temperature and metabolism. Seasonal variation in oxygen consumption of the mound-building ant, *Formica ulkei*, is entirely explainable in terms of the temperature of its nest which signifies that this species hibernates but does not undergo diapause

(86). The oxygen consumption of certain lepidopterous larvae showed the enormous variation from 911 cc./kgm./hour at 28°C. to 10,000 cc./kgm./hour for short exposure at 46°C. (313). Calorimetric determination of the heat produced by the grasshopper, *Gastrienargus transversus*, showed that it varies directly with the temperature of the surroundings (207) which, of course, is directly contrary to the behavior of homoiothermic animals. Data on the effect of temperature upon the respiratory quotient are somewhat contradictory. With grasshopper nymphs and larvae of the Japanese beetle (202), with adult grasshoppers (207), and with the honeybee (100), it is constant over the range of average temperature. But with the mound-building ant the respiratory quotient varies nearly linearly from 0.500 at 4.0°C. (average winter temperature of the nest) to 0.866 at 22°C. (average summer temperature of the nest) (86).

Variations in atmospheric pressure have little effect upon gaseous exchange (139), though the honeybee (434) and the dragon fly larva (162) require some time in which to become adjusted. Consumption of oxygen by the termite, *Termopsis nevadensis*, is not affected by decrease in its partial pressure until a concentration of about two per cent is reached (68). This insect is able to respire normally in atmospheres containing carbon dioxide up to twenty per cent. Higher concentrations cause a reversible anesthesia. Grasshopper eggs respire at the same rate until the oxygen partial pressure falls to 25 mm. (34). Resistance to lack of oxygen is a general characteristic of insects for many species can live for long periods in its absence. Possibly an extreme example is afforded by the larvae of the horse botfly, *Gastrophilus equi*, which normally live in conditions of low oxygen supply within the stomach of the horse and have been reported to live seventeen days without any oxygen (84). Anaerobic metabolism is possible with many species of insects. While this occurs the insect is quiescent and basal metabolism proceeds without the oxidative steps. The accumulation of unoxidized products causes a condition of oxygen debt which usually leads to a very high consumption of oxygen when that element is readmitted. But the termite has been reported to show no oxygen debt after two days without oxygen (68). The readiness with which non-oxidative metabolism occurs shows close correlation with the natural habitats of several aquatic larvae such as *Chironomus* sp. (140). A very interesting observation has been made with grasshopper eggs to the effect that 80 per cent of the respiration of actively developing embryos may be inhibited by cyanide but during diapause cyanide has no effect in small

doses (35). The respiration during diapause is qualitatively and quantitatively identical with the cyanide resistant respiration of the developing embryo. Oxygen consumption increases during development in proportion to the iron incorporated in the embryo (35).

The nutritional condition affects the gaseous exchange of insects. During starvation the respiratory quotient falls just as it does with mammals. Thus after eight weeks that of the mound-building ant decreases from 0.835 to 0.45 which doubtless signifies that carbohydrates are metabolized chiefly at the beginning and proteins or fats at the end (86). Entirely similar results are obtained during starvation of termites (69). During metamorphosis oxygen consumption first falls and later rises, giving a characteristic U-shaped curve which has usually been interpreted as due to corresponding variation in the amount of organized tissue. However, the respiratory quotient is low at the same time, which circumstances may be interpreted to indicate incomplete combustion of fats (229) and the blood is more acidic than during larval life. On the basis of these facts the suggestion has been made that the decrease in oxygen consumption during the pupal period is due to anaerobic metabolism (427d). Heat production also falls sharply during the first part of metamorphosis and to a less extent during each molt (20). Desiccation of the larvae of the mealworm causes an increase in the body temperature which has been interpreted as due to increase in visocity of their protoplasm (17) and thus bears on the old argument of whether temperature coefficients of biological processes are related to protoplasmic viscosity. Dehydration of developing grasshopper eggs by immersion in hypertonic salt solutions brings about decreases in the rate of oxygen consumption which are roughly proportional to the osmotic pressures of the solutions (32).

Blood and circulation. The blood of insects serves as a carrier of food to the cells and waste to the excretory system and in the transport of hormones but as has been pointed out, it is not a particularly efficient oxygen carrier. It is probable, however, that fluid of the tissues serves to carry oxygen from the tracheoles to the nearby cells and that the blood plays an important rôle in the transport of carbon dioxide (420). Most of the recent work has been concerned with the action of the heart and the composition of the blood, although some investigations have been made on other functions.

Circulatory organs. The "heart" or dorsal vessel usually extends the length of the body and has a relatively short aorta although it may be branched and serve to orient the flow in several directions. The

return flow to the several chambers of the heart is guided by membranes and in the wings and legs frequently is aided by accessory hearts (45). The chambers of the heart may be largely non-contractile as individuals. They are separated by the valves of the ostia or semilunar valves, but in some insects, e.g. Ephemerida, these valves have been altered to serve as interventricular valves which prevent the backward flow of blood in the heart itself (269). There are small pulsatile organs situated at the bases of the antennae and of the wings and in the legs. While these organs are not always present, they are found in *Dytiscus* (45) and *Nepa* (Hemiptera) (137).

The heart action. The pumping action of the heart is due to a peristaltic wave of contraction which passes over the heart from the rear forwards. Usually the wave covers the entire heart so that the rate of pulsation of each chamber is the same (45), (213), but in *Cloeon dipterum* (Ephemerida) the ostium in the tenth segment contracts 15 times a minute (269) whereas the ostia in front of this segment contract 60 to 70 times a minute. Although the heart beat usually starts at the rear there are many cases of reversal. In such instances the initiation of the heart beat may occur in the anterior end of the heart, or in the middle or even at both ends at once. There may be a periodic reversal, each period lasting as long as 30 minutes. The rate of beating is the same forward or backward (123). The rate of heart beat varies greatly, from 12 in *Corethra plumicornis* (Diptera) larvae to 140 in *Sphinx ligustri* (Lepidoptera) adult (213). Reversals of blood flow occur in the wing circulation of the cockroach; sometimes the circulation in one wing only is reversed. Reversal of flow may occur with or without reversal of the heart contraction (441). There is no conclusive evidence as to the nature of the control of the heart action. The characteristic heat of activation (μ value of Crozier) has been studied by many workers (364), (206), (435). The value of μ indicates that the heart action is under neurogenic control. Reversal of the heart beat occurs independently of the central nervous system and is assumed to be myogenic in origin (123). The action of the isolated heart has been studied in the case of the cockroach. The time for each beat is variable; systole 0.1 to 0.2 second and diastole 0.2 to 0.5 second when the rate is 60 contractions per minute. The amplitude is lower at the oral chamber of the heart and varies with the rate; at a rate of 60 the amplitude is 0.5 mm. The rate is directly proportional to and changes greatly with the temperature. The heart action is normal at pH 7 and in the saline mixture isotonic with 1 per cent sodium chloride. Acid or base dis-

turbs first the rhythm, then the amplitude, after which failure ensues. If the amplitude has been increased, return to normal is not usually possible. Hypotonic solutions weaken the rhythm giving a long pause phase; in 0.6 per cent NaCl solution the heart action fails. Hypertonic solutions increase the rhythm and destroy its uniformity, and heart action stops at a concentration of 1.4 per cent NaCl (213).

Blood volume. The volume of the blood of the larva of *Bombyx mori* and *Galleria mellonella* have been measured by two methods, first by opening the larvae and absorbing the blood on filter paper and weighing, and second by washing out the blood, evaporating the blood and washings to dryness and weighing. The blood volume is calculated from the density in the first case and from the content of total solids in the second. The methods give 0.29 cc. per gram body weight for *B. mori* and 0.41 cc. per gram body weight for *G. mellonella* (320). The blood volume of the cockroach has been determined by a corpuscle dilution method giving a value of 0.69 gram per gram body weight (439).

Blood composition and pH. The color of insect blood is usually somewhat green or yellow. The green is not simply due to pigments from the plant food eaten, but is caused by breakdown products of protein metabolism. The yellow may be caused by carotene which is an essential foodstuff. The possibility that it is synthesized in animals, including insects, has been disputed (268), (443). The nitrogenous constituents are present in different proportions than in mammals. Thus in the blood of *Deilephila euphorbiae* larvae the nitrogen is distributed as follows: total 1180 mgm. per cent (i.e., 1180 mgm. per 100 cc.), protein nitrogen 825 mgm. per cent, non protein nitrogen 355 mgm. per cent, amino nitrogen 170 mgm. per cent, peptide nitrogen 120 mgm. per cent, urea 20 mgm. per cent (151), (71). In the cockroach the non protein nitrogen is about 360 mgm. per cent, and is markedly decreased by arsenic ingestion (289). There is some evidence that toxic gases produce only slight changes in insect blood (353). Urea is always present but in small amounts (80). The blood of the silk worm contains 152 mgm. per cent of tryptophane (247). The inorganic constituents of the blood of *Deiliphila euphorbiae* larvae are inorganic phosphorus 12 mgm. per cent, calcium 41 mgm. per cent, magnesium 43 mgm. per cent, Cl 48 mgm. per cent, and iron 5.8 mgm. per cent (151). The copper content of insect blood is less than that found in other tissus (259). The blood of the honeybee contains 190 mgm. per cent of phosphorus (182).

The pH of the blood is about the same as in mammals but is not so constant. In the adult honeybee worker the pH is 6.7 (182). In *Sceliphion caementarium* (Hymenoptera) the pH varies: larval 6.6 to 6.8, prepupal 6.3 to 6.5, pupal 6.3 (41). In the silk worm the pH varies from 7.2 in the egg to 6.8 in the larva and 6.7 in the pupa and adult. There is a sharp rise and fall of about 0.15 pH during each molt (81).

The blood of *Bombyx mori* contains tyrosinase, catalase, phenolase, sucrase, maltase, lipase, and two proteases (436). The differences in optimum conditions of temperature and pH for these enzymes are as follows: tyrosinase, 37°C., pH 6.6; catalase 23°C., pH 6.6; phenolase (oxydase) 40°C., pH 6.6; phenolase (peroxydase) 20°C., pH 6.6; sucrase —, pH 6.6; maltase —, pH 6.6; lipase 40°C., pH 7.7; protease A —, acid; protease B —, basic. The concentrations of these enzymes vary with age and stage of development (437), (438).

In the cockroach, coagulation of the blood is not accompanied by the formation of a fibrin network. In drops of blood submerged in oil the clotting is seen to be due to change in the cells which form thread-like pseudopodia, clump and finally disintegrate, leaving a semi-solid mass. There is little change in the plasma. Coagulation may be prevented by heating the insect to 60°C. for 10 minutes (439). The types of coagulation of blood in seven orders of insects have been investigated. There are three types: *a*, no plasma coagulation and negligible cell coagulation; *b*, slight plasma coagulation and marked cell coagulation; *c*, marked plasma coagulation and some cell coagulation. There is no relationship between classification of the insect orders and the type of coagulation found (440).

Cellular constituents. The appearance of the blood cells or hematocytes is markedly variable depending upon the age and condition of the insect. This has led many of the earlier workers to assume the existence of several types of cells with different functions. It is desirable to designate the separate forms whether of different origin or merely of different age. It is very necessary that some definite nomenclature be adopted and the cell-types characterized so that they may be recognized in different insects.

The blood cells are sometimes ameboid and are free or fixed in position (424). They may or may not be phagocytic and the size of the nucleus varies. The phagocytes frequently collect in great numbers about a foreign body and may encapsulate a parasite. There is some evidence that the phagocytic cells of insect larvae will not encapsulate a parasite unless tissue injury occurs (383). It is possible that they

play an important part in connective tissue membrane formation (323). Smaller foreign bodies such as bacteria are completely engulfed by the cells (96). In chironomid larvae certain species have only free phagocytic cells while others may have some or even all of these cells fixed to other tissue elements forming a phagocytic organ (224).

The house fly fed on ammonia carmin dissolved in sugar solution develops syncytial masses of the polynuclear phagocytes which are most numerous between the pericardial cells (365). In several caterpillars the phagocytes are able to digest injected tubercle bacilli (179). The large blood cells or amebocytes of the cockroach have been studied in vivo and in vitro. In the roach the number of cells increases during unfavorable conditions but in greatly weakened or old insects they become very few in number. The number of mitoses observed in tissue culture suggests that the rate of cell division cannot account for the rapid increases in numbers and that they must arise in some other tissue (379).

THE NERVOUS SYSTEM AND SENSE ORGANS. *Central nervous system.* While a great deal of research has been done on the isolated nervous system of cold-blooded vertebrates comparatively little work has been done on invertebrates and almost none on insects. It has been shown that the rhythmic muscular movements during respiration in *Dytiscus marginalis* (water beetle) correspond with a rhythmic discharge in the efferent fibers to these muscles (2). These discharges are also present in the isolated nerve system and are presumed to be due to a progressive depolarization and recovery in the dentritic region of the ganglia. The contractions of the body muscles of *Dytiscus* are due to trains of motor impulses which rise and decline in frequency in a manner similar to the action in vertebrates. The action of nervous system poisons and anesthetics seems to be progressive from the periphery to the central in the case of the house fly (161) in which formaldehyde, ether, and chloroform cause a progressive paralysis from posterior to anterior. The recovery occurs in the reverse order.

While the brain does not have so important a function in insects as in higher animals it does exert a controlling or inhibitory influence on the reflexes (313). The reflex paths have been scarcely studied but some work has been done on peripheral inhibition (118) and nervous coördination (113). The respiratory movements are initiated by an inherent nervous rhythm (2) but this is greatly influenced by inhibitions imposed by external stimuli (113). The primary centers lie in the segmental ganglia and the secondary or coördinating center lies in the prothorax (420). Decapitation does, however, have some effect

(313). The visual center is located in the tritocerebrum and is (12) presumably also the nervous center concerned in morphological color changes in the stick insect, *Dixippus morosus*.

Sensory organs. Much of the work done on the sense organs concerns two aspects, the morphological and histological structure and the response of the organism as a whole. The connection between structure of the organ and the response, the physiology of the organs, has been neglected. One of the important functions of the several sensory receptors is that of stimulating (429) the organism and increasing its awareness of its surroundings. This function has been attributed to the halteres of Diptera (397), the antennae of *Rhodnius* (425) and the ocelli of Lepidoptera (117). Overstimulation of the sense organs may lead to thanatosis or the imitation of death. If the stimulation be often repeated thanatosis finally become inhibited (212).

A stimulus may cause certain types of activity, as in the effect of light on the diurnal rhythm in the Orthoptera (234) and on the synchronous flashing of fire-flies (316). Since the response of the organism to visual and chemical stimuli is the most definite, the photo- and chemo-receptors are the best understood. The photo-receptors are usually located on the head and are simple eyes or ocelli and compound eyes, but the whole integument may be light sensitive (368). Although the simple eyes are probably incapable of image formation, those of the Cicindeladae larvae are able to perceive movement (117) and thus aid in locating prey, and those in *Lucilia sericata* larvae are not only extremely sensitive to light, but can be extended and withdrawn at will (93). The ocelli of adult insects which also possess compound eyes function differently from those of larvae, since if the compound eyes of bees or of ants are covered over, the insects are no longer able to orient to light (280).

The chemoreceptors are apparently located on various portions of the anatomy of the insect. While the antenna usually carry most of the chemoreceptors (273) large numbers of them have been found on the tarsi of Lepidoptera (94). It is very difficult to differentiate between receptors for odors and those for taste, i.e., sensations perceived by the human nose and tongue respectively. Thus the taste of salt is detected by receptors situated in the mouth parts of the honeybee (119), on the tarsi in Diptera and Lepidoptera and on the palpi of some Coleoptera (411). The sense of smell is usually restricted to the antennae, but may also be present on the wings, elytra, legs and mouth parts in Coleoptera (238). In the case of the male mealworm beetle, it has

been found that the same portion of the antennae responded to the odor of the female and to food (404). The structure of the sensilla varies somewhat but no correlation between structure and function has been found. Apparently similar organs function as receptors for taste and for odor. The nerve fibers are probably not exposed to the air (288) but are covered by a readily permeable membrane.

In the few cases studied some insects appear to have special receptors for heat. In *Rhodnius prolixus* (Hemiptera) (425) these are situated mainly in the antennae while in *Schistocerca gregaria* (Orthoptera) the sensilla are distributed over all the body (111). There is no indication that these receptors are essentially different from those receiving other stimuli.

The auditory organs of insects have been studied from both the physiological and the observational view points. The actual nerve impulses originating in the tympanal organ of crickets and katydids have been studied. The organ responds to sound frequencies from 250 to more that 11,000 vibrations per second. The quality of the response does not vary with the frequency (408). That insects respond to sounds is well established although some investigators have thought that the response was due to vibrations perceived through the support upon which the insect rested. There is now considerable evidence that certain insects respond to sounds transmitted by air and received by special auditory organs (14).

The auditory responses of Orthoptera and Coleoptera have been studied by such methods as amputation of the organs under local anesthesia and electrical transmission of the sound. Many species of the orders Orthoptera, and Coleoptera, and at least one species of Hymenoptera, Rhynchota and Lepidoptera respond to sounds. Sounds emitted by certain insects may serve as mating calls and as means of orienting each individual to the location of the others. In some cases the type of sound is correlated with pleasing or disagreeable sensations such as foods, enemies, etc.

All insects lack, so far as is known any specialized statocyst organ and response to a gravitational field is by means of tension receptors or the kinesthetic sense (160). That such afferent impulses exist has been shown for the cockroach by means of unpolarized electrodes (15).

Perception and response to stimuli. Since all experiments on lower animals are necessarily objective in nature it is always dangerous to admit of volition on the part of the organism. This has led many workers to a mechanistic concept of the underlying forces governing

the response to stimuli. The three principal theories of insect response are Loeb's tropotaxis, and Kuhn's telotaxis and menotaxis. In tropotactic response it is assumed that the muscle tonus of the locomotor appendages is controlled by the strength of the stimulus received by the sensory perceptor; thus the organism is oriented by the differential intensity of stimuli received by bilaterally symmetrical receptors. This explanation of response may be applied to the orientation of muscid larvae (93). If a honeybee is given training, it will learn to choose one of two equal light sources and travel directly toward it; if blinded on one side, or if the legs on one side are shortened the bee may still learn to follow a direct path toward the light (398). This result does not follow the theory of tropotaxis and from the nature of the response is termed telotaxis. The telotactic reflex may be inhibited by higher centers as in the bug, *Rhodnius*, which responds much more readily to moving objects when the antennae have been removed (425). Certain insects move at a constant angle to a source of light, the response being termed menotaxis. This may result from the effect of two stimuli one from the light and the other from the background (74) or it may be caused by a change in the number of ommatidia stimulated due to light adaptation (73). The "light compass" movements of many insects may be included under menotaxis (428). It is difficult to apply the idea of tropotaxis to the response to other stimuli such as dissolved substances (taste), odors and sound. In telotaxis there is a movement in the direction of optimum stimulation, governed in the case of light, either by the strength of the stimulus or by the number of receptors. In the case of odors there is the same effect, that is, a "reflex pursuit of the antenna into a region of optimum stimulation" (425).

Reaction to light and heat. The visual acuity of the honeybee has been extensively studied (150), (430), (431), (432), (159). As in man, the visual acuity increases with intensity of illumination until a constant maximum is reached. The maximum acuity for bees is about one hundredth that of man, i.e., the bee's eye at its best efficiency is about equal to man's at its poorest. The ommatidia are not equally sensitive to light, so that at low intensities the eye behaves as if it had fewer ommatidia and hence the visual acuity is determined by the size and angular separation of the elements. The ommatidia are smaller near the center of the eye and therefore the visual acuity is better in that region. The ability of the bee to recognize objects and patterns has been found to conform with its visual acuity.

The color vision of insects has been intensively studied both in the

laboratory and in the field especially in connection with light traps. The honeybee has two maxima in stimulative efficiency in the spectrum, one at 553 μ in the yellow-green region and one at 365 μ in the ultra violet region. The stimulative effect of the ultra violet maximum is about 4.5 times that of the visible (18). *Drosophila* flies show the same type of color vision, having the maxima at 487 μ and 365 μ, the ultra violet maximum being 5 times as great as that of the visible (19). In field tests it has been shown that the codling moth and the artichoke plume moth are much more attracted to blue, but the grape leafhopper is more attracted to red lights. The ratio of female to male grape leafhoppers attracted was 7:1 for blue, 1:14 for red and 2:3 for white lights (158a). Strong white light has a repellent effect upon the codling moth or a deterrent effect upon egg laying as indicated by lower infestation of apples within an artificially lighted area (158). The attractiveness of lights to insects is considerably influenced by temperature, 60°F. being the lower limit for the codling moth (298), (99) and leaf roller (66).

The heat receptors may lie in the antenna as in *Rhodnius* (425) or over the entire body as in the locust, *Schistocerca gregaria* (111). The biting fly, *Lyperosia exigua*, and the bed bug, *Cimex lectularius*, both respond to temperature differences (216), (325) but *Cimex* probably does this over only a very short distance (199).

Olfactory and gustatory responses. Two methods of determining the olfactory response are available, one in which a confined population or individual chooses between two odors and the other in which bait traps are used. It is very difficult to estimate relative attractiveness by the latter method and, of course, it is impossible to determine repellency except by indirect methods. The principles involved in designing an olfactometer have been discussed (183). In general the attractive odors are those associated with sex or with food, either of the individual tested or of the larva of the species if the individual is a gravid female, seeking a suitable place to oviposit. Thus decaying meat is attractive to blowflies (239) and the odor of the skin and its secretions is very attractive to blood sucking insects (215), (325). There is some correlation between attractiveness and chemical constitution as is evidenced by tests with pure compounds. Fatty acids are attractive to wire worms (227) but the attractiveness of essential oils to the cockroach is not so well correlated with structure (65). The concentration is also important, since some substances attractive at low concentrations become repellent at high concentrations, e.g., methyl-

amine and ammonium hydroxide to the cotton boll weevil (107) and oil of peppermint to the cockroach (65). The sex attractants are usually volatile secretions formed only by the female (284), (404), (67) but may be simple substances such as fatty acids (227).

In testing for gustatory responses one must usually rely upon feeding responses which may be elicited by odors, e.g., the proboscis response of Diptera (1). The sensitiveness of the response depends upon the degree of inanition of the insect, upon the species and upon the substance. The monarch butterfly responds to 10^{-5} M saccharose solutions, while the red admiral butterfly is sensitive to 10^{-2} M saccharose (7). The receptors for certain substances may be localized. Thus in the blowfly, *Calliphora vomitoria*, the oral lobes are less sensitive to saccharose than the legs, but are sensitive to lactose whereas the legs are not (272). There is no apparent relation between chemical structure and attractiveness (119).

PHYSIOLOGICAL RACES. Among the various lines along which insect physiology has developed in recent years one of great interest is that concerned with races which are isolated more or less completely by physiological barriers. A comprehensive review of the subject up to 1930 has been given by Thorpe (385), who has defined a physiological or biological race, as he prefers to call it, in the following words: "A biological race may be said to exist where the individuals of a species can be divided into groups, usually isolated to some extent by food habits, occurring in the same locality and showing definite differences in biology, but with corresponding structural differences, either few and inconstant, or completely absent." A large number of examples of insect races, both naturally occurring and experimental, are listed and discussed.

Food preferences. A number of groups recently studied may be considered as races with different preferences for food or oviposition. The interesting suggestion has been made that this situation may be considered to arise from a "conditioned reflex" since the act of oviposition by the adult female may be influenced in some psychological manner by the fact that as a larva she fed upon the material in, or on, which the eggs are laid (403). The fact that the central nervous system remains more intact than any other important part of the body may be considered to provide a physical possibility for such a psychic association. In Scotland two races of the apple sucker, *Psyllia perigrina*, are found, one on apples and the other on hawthorn. They do not interbreed and have entirely different parasites, except for one dipteron, *Endopsylla* sp. (222). A study of the migration of a number of species of erioso-

matine aphis has shown that several of these exist in two races, one of which has an obligatory alternate host—e.g., gooseberry during the summer—while the other can continue the life cycle exclusively on the elm (253). An example of the transference of induced food habit to offspring is given by *Caransius morosus* (Phasmidae). Individuals from a stock reared for many generations on privet were induced with difficulty to feed upon ivy. The succeeding generation showed a decided preference for ivy (358). This provides another example of the already long list of insects which have changed their feeding habits either with or without the intervention of man.

On the basis of difference in reaction to temperature and humidity, two races of the parasitic hymenopteron, *Trichogramma minutum*, have been distinguished (233). Differences in pigmentation and in effect of temperature upon rate of development have been used in distinguishing at least four races of this species (108). A study of nine "strains" of *T. minutum* from the West Indies and of two from North America showed marked differences in host preference and possibility for crossbreeding (138). They also differ in type of parthenogenetic reproduction. Two races of the Mediterranean flour moth, *Ephestia kühniella*, differ in susceptibility to control by a braconid parasite because their lengths of life cycle differ (299).

Races of mosquitoes. It has been mentioned previously that occasionally the mosquito, *Culex pipiens*, lays eggs without having taken a blood meal previously. Largely on the basis of this behavior it has been claimed that two races exist in France. A "normal" one predominates in rural districts, requires blood before it can lay eggs and passes through a hibernation each third generation. The "autogenous" race lives mostly in cities and towns, breeds continuously and lays eggs without a blood meal (329). Larvae of the autogenous race are much more resistant to ammoniacal products of protein decomposition (331). The idea of hibernation as a period of "physiological purification" has been revived in connection with *Culex pipiens* (334). The existence of two races has been denied on the grounds that ability of the adult female to lay eggs without partaking of a blood meal is entirely a matter of her larval nutrition and that in consequence of inadequate food as a larva the adult female attacks man to obtain nourishment to sustain life (40). Attempts to distinguish races of *Culex pipiens* on morphological grounds have not been conclusive, and the whole question is as yet unsettled, probably because its practical importance seems to be small.

Quite the contrary condition prevails with reference to the mosquito,

Anopheles maculipennis, which is the most important carrier of malaria in Europe and in certain other parts of the world. The occurrence of "anophelism without malaria" in numerous districts led to the suspicion that races might exist, varying in ability to transmit the malarial organism, in adult food preference, in climatic preferences, and in many other ways. A tremendous literature has arisen in recent years, most of which is mainly medical or ecological and is not pertinent here. A good summary up to 1932 has been given by Hackett, Martini, and Missiroli (135). A very complete discussion and bibliography on physiological aspects of the question was given the following year by Hecht (149). Two races were first recognized which differ in markings on the eggs and in habitat. *A. maculipennis labranchiae* lays a dappled gray egg, is more prevalent in human dwellings, does not go into complete hibernation in the winter, and occurs wherever malaria is prevalent. The larvae tolerate brackish water. *A. maculipennis messeae* lays an egg with two transverse black bars, frequents stables, hibernates in winter, and predominates in non-malarial districts. Its larvae prefer fresh water. Differentiation on the above bases as stated in 1932 (135) has in general been confirmed (51) but not unanimously—e.g., the maxillary index, i.e., number of teeth, has been urged as a better criterion than egg marking (333). The chloride content of breeding water does not always correlate with race (412). Studies in Russia indicate the presence of two races having different optimum humidities for biting humans (16). Recent work indicates five races of *Anopheles maculipennis* or six if *A. sacharovi* (*elutus*) is considered as a member of the race complex (274). Of these *labranchiae* and *sacharovi* are most important as malaria carriers, *messeae* and *atroparvus* ordinarily are restricted to feeding upon animals but may attack man if other food supply is not adequate, while *melanoon* and *typicus* (which is also called by various other names) are of practically no importance (136).

Resistance to toxic substances. Division into races has been made with a few insects on the basis of varying susceptibility to control by insecticides. Larvae of the Colorado race of codling moth, *Carpocapsa pomonella*, reared under Virginia conditions for several years, have maintained their superiority over Virginia larvae in entering fruit sprayed with lead arsenate and several other stomach poisons (184). But direct injection of sodium arsenite and acid lead arsenate solutions into the hemocele revealed no difference in susceptibility between larvae from Colorado, Virginia, and Missouri (144). In southern

California a strain or race of the red scale, *Aonidiella aurantii*, first discovered by Quayle in 1916 to be resistant to hydrogen cyanide has been spreading steadily for several years and has greatly complicated the problem of control in the citrus districts although proper dosage and careful application enable fair control to be obtained (277). A similar situation exists with the black scale, *Saissetia oleae*, and the citricola scale, *Coccus pseudomagnoliarum*. It would be hard to overstate the importance of such races of injurious insects from a practical point of view. A discussion of their possible significance in evolution has been given by Thorpe (388).

Phases of locusts. The recent work on the phases of certain locusts may be considered at this time although it does not have to do with insect races. The tremendous migrations of locusts, which occasionally occur, were explained by Uvarov in 1921 on physiological grounds. He designated the migratory phase of these insects as *gregaria*, referring to their habit of assembling in great numbers, in contrast to the phase *solitaria*, which does not migrate. The great activity during migration was postulated as a necessity for completion of sexual development of the phase *gregaria*. The eggs also undergo a diapause. This explanation of the cause of migration has been disputed and the psychological factor of "social influence," as well as the morphological differences, have been attributed to conditions of dispersion or of crowding, which in turn are dependent on environmental factors that control the number produced (375). Laboratory rearing experiments have shown that progeny of *Locustana pardalina solitaria* can be transformed into the phase *gregaria* by crowding during the early instars (101). Similar experiments have shown that *Melanoplus spretus* whose migrations did great damage in the central states many years ago is the gregarious phase of M. *mexicanus* and should be called *M. mexicanus gregaria* (102).

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VARIATIONS IN WHITE BLOOD CELL COUNTS

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The interest in this review has centered primarily about the total leucocyte count and its variations under different physiological conditions. Of necessity certain aspects of the differential count have been considered but its broader presentation is beyond the scope of our purpose.

Blood counting had attracted the interest of Nasse prior to 1842 when he wrote a section on this subject in Wagner's *Handwörterbuch der Physiologie*; likewise Vierordt and Welcher each referred to the procedure in their works published in 1854. This was the year Moleschott noted the greater variability of the counts of white cells than of red cells, which gave rise to the concept of a digestive leucocytosis long considered the chief source of variation of the number of white cells.

The various steps in the development of the modern technique have been given detailed description by Bürker in Tigerstedt's *Handbuch der Physiologischen Methodik* (II_2, 1913). It is chiefly a development of precision in the pipette introduced by Potain (1867) and in the cross-ruled chambers devised by Welcher (1854), by Abbé (1878) and Thoma (Lyon and Thoma, 1881).

Few changes in the standard methods have been introduced in recent years and these chiefly to satisfy personal preferences of the investigators, thus Osgood and Haskins (1931) use oxalated venous blood; Ellermann and Erlandsen (1910) use 0.1 cc. of blood mixed with a diluent in a small test tube—a method recently recommended by Krogh (1929)—and Piney (1924) developed a special pipette which has been employed by Purdy (1925) in a study of technical errors. In our experience none of these special methods has any advantage over the commonly employed clinical methods when the latter are properly used.

One working in this field is soon impressed with the variability of successive counts made on a given subject. Such variations as are the result of true physiological fluctuation can be detected or evaluated

only if the inaccuracies due to technic are reduced to a minimum. The technical sources of error arise from *a*, failure to procure samples representing systemic blood; *b*, failure to properly mix the diluted sample in the pipette and thus to secure uniform distribution of cells in the pipette and on the field of the counting chamber, and *c*, failure to conform to the old admonition to count many cells and thus minimize the inequalities of distribution on the field of the hemacytometer. It should be appreciated that any study of physiological variations in the number of the blood leucocytes should concern itself with the cells in the circulating blood and not with accumulations of cells in various organs. We are practically limited in a study on man to samples of blood obtained from veins or to so-called "capillary blood" obtained from punctures of the skin of the fingers or ears (cranial sinuses or heel in the case of infants). Shaw (1927) found no difference in leucocyte counts between arterial and venous blood in human beings and concluded that capillary blood, when it was obtained after frictional vasodilatation or warming, gave the same leucocyte counts as arterial blood. Merely by waiting for the initial vasoconstriction to pass off and using later drops after the vasodilatation has set in is equally satisfactory as shown by Bryan, Chastain and Garrey (1935a). The latter authors wipe off intermediate drops with gauze moistened with oxalic acid to prevent the initial stages of clotting. Krogh (1929) has discussed the mechanism of blood flow from stab wounds made to obtain samples of blood for counting and points out that "the blood will flow mainly from arteries which have been cut while the capillaries and venules will contribute only an insignificant amount." He states that consistent results can be obtained when an active hyperemia is produced in the ear with a very sharp instrument giving a minimum stimulus toward the contraction of the arteries.

The mixing of the blood in the pipette after adding diluting fluid (e.g., Türk's solution, 1902) requires special care; it is best accomplished by the method recommended by Potain or some modification of it such as that of Bryan and Garrey (1934) which employs a mechanical "rotor." The principle involved is merely a moderately slow rolling movement of the pipette while alternately tilting its ends up and down. After this procedure the counting of samples from every drop in a pipette will give a lower coefficient of variation than by other methods. The advantages of counting a minimum of 800 cells on the field of the hemacytometer have recently been emphasized by Ponder, Saslow and Schweizer (1931); this alone will not suffice to offset the necessity for observing the other technical precautions mentioned above.

Analyses of the sources of error have been made by "Student" (1907), Purdy (1925), Ponder, Saslow and Schweizer (1931), Bryan and Garrey (1934) and Bryan, Chastain and Garrey (1935a). The latter authors have pointed out that significant physiological variations represented by counts of different magnitudes may be overlooked unless the technical errors represented in the differences are properly expressed, and this may be done more accurately by using absolute scales such as standard deviations, probable errors, etc., in actual number of cells, than by stating them in terms of percentages, since the former do not vary appreciably within the physiological range, while percentages, e.g., coefficients of variation, are in part a function of their respective means and due allowance must be made for this fact.

Although the literature dealing with the variations in the leucocyte count is replete with contradictions concerning causes, conditions and extent of variations yet it is noteworthy that the limits within which practically all (say 95 per cent) of the variations are observed are relatively fixed. It is well established that excessive physiological activity as well as disease may alter the leucocyte content beyond the usual limits so that there is often a gradation between normal and diseased states, such that it becomes difficult, in fact impossible in many cases, to draw a clear cut line which delimits the normal from abnormal states. To merely state the average or the upper and lower limits for the population as a whole is of little significance. Of far greater import is the frequency distribution of the leucocyte counts of a large bulk of the population. Such a frequency distribution includes the frequency distributions of individuals each of which has a more limited range and a level which is more or less characteristic of the individual. Some counts will fall near the lower limit, some near the upper limit of the normal physiological range. The significance of any single count can be judged only according to the frequency with which counts of this magnitude occur in a population of normal individuals. The enormous literature on leucocyte counts indicates clearly that as the leucocyte count increases above 10,000 cells per cubic millimeter of blood the chances of its occurrence in a normal individual decrease rapidly (cf. fig. 2) and for this reason this arbitrary number has been set, quite universally, as designating the upper limit of variation in normal adults. One must not overlook the fact that there are individuals, however, in whom the frequency distribution shows an upper limit much below this figure which, for them, would be distinctly in the region indicating a pathological leucocytosis. Lacking the possibility of having a frequency distribution for every individual we are forced for our clinical

new born may rise for the first two months the tendency for a gradual decrease in the leucocyte count for the first two years of life is quite generally reported. Washburn (1934) has reviewed the literature in which he found variations in the count from a low 3,800 to a high 23,000. The greatest variation found in his own series was from 8,800 to 23,150. The counts of individual infants were pitched at different levels, however, and tended to fluctuate within definite zones which were characteristic of the individual, the zones were not necessarily the same for any two infants of the same size or age. These findings have been confirmed by McBryde (personal communication, 1935). The reports by many authors fall within the limits published by Lucas (1930); these limits are: sixth month 10,000 to 15,000; first year 9,000 to 14,000; second year 8,000 to 13,000 with an average for the two year period of 11,000. The gradual decrease in limits and in averages with increase in age should be noted but the variation for averages does not amount to more than ±1,000 cells. Lymphocytes predominate throughout this period but neutrophiles and lymphocytes are present in practically equal numbers at the age of two years (Lucas, 1930; Blackfan, Baty and Diamond, 1930; Benjamin and Ward, 1932; Holt and McIntosh, 1933). Lucas (1930) gives the limits of normal variations in cell types during the second year of infancy as follows: polymorphonuclear neutrophiles 35 to 55 per cent, lymphocytes 35 to 55 per cent, endothelials 3 to 8 per cent, eosinophiles and basophiles 0 to 4 per cent.

Childhood. The limits of variation of the total leucocyte count and the type of cells in the differential count for the period of childhood ending with puberty, vary with age. The lack of uniformity in the age groupings reported by different authors is such that an adequate summary is impossible and the reader is referred to the published reports of Blackfan, Baty and Diamond (1930), Lucas (1930) and Benjamin and Ward (1932). In 1934 at the Cleveland meeting of the American Medical Association, E. E. Osgood exhibited charts and reported "Normal Haematological Standards" based on an extensive study of different age groups between the ages of four and thirty years. For three groups representing the ages of 4 to 7, 8 to 18, and 19 to 30 years he gave the means for the total leucocyte count for the respective groups as 10,400, 8,300 and 7,400; and in the same order the limits of the counts were given as 6,000 to 15,000, 4,500 to 13,500 and 4,500 to 11,000; these are the limits which include 95 per cent of the individual counts. They show the tendency of both maximal and average leucocyte count to fall as adult age is approached. Osgood's data, which

were generously submitted to us by him, have been compared with data which include the leucocyte counts on 500 supposedly normal children between the ages of 5 and 19 years. The latter data were secured during a survey of hookworm conducted by the Rockefeller Foundation and directed by Dr. A. E. Keller who kindly placed the findings at our disposal. Our comparison has been made in the form of the frequency distribution curves shown in figure 1. The two distributions are strikingly similar and their significance will be discussed in relation to similar distribution curves constructed for adults. Both series of counts on which the frequency curves for children have been constructed were made on venous oxalated blood, as recommended by Osgood and Haskins (1931) and the studies directed by Keller added the use of the rotor device of Bryan and Garrey (1934) for mixing cells and diluent.

Adults. The magnitude of leucocyte counts of normal adults, if taken without regard to time of day or physiological conditions which are known to cause variations, show a fairly wide range. Galambos (1912) reported a range from 3,500 to 12,500, Feinblatt (1923) from 6,200 to 15,600, Fairley (1923) from 4,500 to 15,000, Sabin, Cunningham, Doan and Kindwall (1925) from 4,200 to 13,680 and Osgood (1934) from 4,400 to 13,250. In a new series of 627 observations on 200 subjects under random physiological conditions Bryan, Chastain and Garrey (1935a) found limits from 2,700 to 14,000. Relatively few reports have been presented in such a way that one could determine the frequency with which a given count, within the normal range, would occur in a normal population. We have constructed cumulative frequency curves, or ogives (fig. 2) which represent the published data of a number of authors who have studied the total leucocyte count in normal adult individuals; the studies by Shaw (1927), by Schweizer (1933) and by Bryan, Chastain and Garrey (1935b) were made during rest in the early morning. In the studies of Galambos (1912), and E. E. Osgood (1934) samples of blood were taken at random during the day but those of Torday (1913) were made on fasting subjects in the early morning hours (9 to 10 a.m.). This type of frequency curve has been selected since the character of the curve is not fundamentally altered by differences in class interval groupings, which vary with different authors, and it is particularly well adapted to interpolation. Thus if it is desired to find the value of the leucocyte count below which 50 per cent of the observations lie (i.e., median) or below which 95 per cent of the observations lie (i.e., 95th percentile), these values may be

judgment of a single blood count to fall back upon a frequency curve of the population, which curve includes all elements of variation that cannot be easily and readily controlled or classified. The importance of such a normal standard was recognized in a series of studies by Pearce and Casey (1930) who, previous to their studies of experimental disease in rabbits, made extensive studies of the blood of normal rabbits, determining not only the total frequencies of leucocytes but also frequencies of the different types of leucocytes and of the red blood cells and the hemoglobin. Similar statistical studies were previously reported by Bushnell and Bangs (1926) who made a large number of observations but only on a very few rabbits. Since the studies on the cytology of rabbit's blood by Brinckerhoff and Tyzzer (1902a and 1902b), who reviewed all the leucocyte studies on rabbits published previous to their work, a large literature has accumulated which includes the investigations of many different diseases and variations of physiological state. Cheng (1930) in his report of extensive studies on the influence of various physiological factors and pathological conditions on the leucocyte count on rabbits has made an exhaustive review of the literature which includes not only references to the investigations on rabbits but also similar studies on human beings. Thus the rabbit has come to be established as a ready animal for the study of the white blood cell picture under different experimental conditions.

Unfortunately the frequency distributions so carefully determined for experimental animals are not applicable to man. The range of normality for human beings can be determined only on human beings. A few such frequency distributions for a normal population have been published by Torday (1913), Shaw (1927), Bryan, Chastain and Garrey (1935b) and detailed counts permitting the construction of frequency curves have been recorded by Galambos (1912) and by Schweizer (1933). Medlar (1929) gives frequency curves representing 241 counts on 17 different individuals, although many of the counts were taken during transient mild infection, e.g., abscessed tooth, cold, sinusitis and so forth. In considering this matter we were faced with the further probability that the frequency distribution for man is not the same for all groupings but varies with certain conditions which can be easily classified and it therefore makes for greater accuracy of judgment of the individual observations to establish different normal standards for each group or class of individuals. For this reason we present the usual groupings on the basis of age.

New born. The generally high leucocyte count of the new born

infant was known to Bayer (1881) and Hayem (1889). Confirmation of this fact is ample in all recent literature and counts as high as 45,000 are recorded by Lucas et al. (1921) and by Forkner (1929). There is a wide range of variation in individuals but a great majority fall below 25,000 and above 10,000, although much lower counts are recorded by Deluca (1927) who reports a minimum as low as 3,600. Curves of frequency distribution have not been published so far as we can determine and the presentation of reports dealing with averages and limits does not permit our presentation of data in this form. The reported averages for the first two weeks of extra-uterine life vary widely; Forkner (1929) reports an average of 24,945 for the first day, while on the fifth day the low average of 8,500 is reported by Gundobin (1893). McBryde in a personal communication (1935) has submitted figures showing high counts of 27,800 and 24,000 and a low count of 4,800. It has been suggested that the white cell count immediately after birth is apt to be higher when the labor has been prolonged, although we know no studies to prove this point; it is reported, however, that the erythrocyte count is raised by protracted labor (Horvath and Hollósi, 1935). There is general uniformity in the report that the white cells remain high and may even increase during the first day or two with a general decline thereafter to the normal level of infancy and childhood; notable exceptions to this rule are met with, however. The leucocytosis is initially due to a preponderance of polymorphonuclear neutrophiles, mainly with single and double lobed nuclei (Sanford, 1929b; Lucas, 1930; Blackfan et al., 1930), trilobed forms appearing about the ninth day to two weeks. By the end of the second week lymphocytes are present in excess as they are throughout infancy (Perlin, 1903; Schloss, 1910; Lippman, 1924; Sanford, 1929a; Forkner, 1929; Blackfan, Baty and Diamond, 1930; Smith, 1930; Lucas, 1930; Holt and McIntosh, 1933). An extensive bibliography of the morphology of the blood of infants is given by Lippman (1924) also, cf. Naegeli, p. 244. Smith (1930) working with the supravital method of Sabin (1923) confirms the later preponderance of lymphocytes which is not so marked as indicated by the fixed smear method. Hall (1930) in a critical review of literature dealing with the supravital staining method rejects the conclusions drawn therefrom but its advantages are strongly upheld by Sabin, Cunningham, Doan and Kindwall (1925), Rogers (1928), Lewis and Shope (1929), Forkner (1929), Smith (1930), Hamil (1931) and Benjamin and Ward (1932).

Infancy. With a few exceptions in which the leucocyte count of the

new born may rise for the first two months the tendency for a gradual decrease in the leucocyte count for the first two years of life is quite generally reported. Washburn (1934) has reviewed the literature in which he found variations in the count from a low 3,800 to a high 23,000. The greatest variation found in his own series was from 8,800 to 23,150. The counts of individual infants were pitched at different levels, however, and tended to fluctuate within definite zones which were characteristic of the individual, the zones were not necessarily the same for any two infants of the same size or age. These findings have been confirmed by McBryde (personal communication, 1935). The reports by many authors fall within the limits published by Lucas (1930); these limits are: sixth month 10,000 to 15,000; first year 9,000 to 14,000; second year 8,000 to 13,000 with an average for the two year period of 11,000. The gradual decrease in limits and in averages with increase in age should be noted but the variation for averages does not amount to more than ±1,000 cells. Lymphocytes predominate throughout this period but neutrophiles and lymphocytes are present in practically equal numbers at the age of two years (Lucas, 1930; Blackfan, Baty and Diamond, 1930; Benjamin and Ward, 1932; Holt and McIntosh, 1933). Lucas (1930) gives the limits of normal variations in cell types during the second year of infancy as follows: polymorphonuclear neutrophiles 35 to 55 per cent, lymphocytes 35 to 55 per cent, endothelials 3 to 8 per cent, eosinophiles and basophiles 0 to 4 per cent.

Childhood. The limits of variation of the total leucocyte count and the type of cells in the differential count for the period of childhood ending with puberty, vary with age. The lack of uniformity in the age groupings reported by different authors is such that an adequate summary is impossible and the reader is referred to the published reports of Blackfan, Baty and Diamond (1930), Lucas (1930) and Benjamin and Ward (1932). In 1934 at the Cleveland meeting of the American Medical Association, E. E. Osgood exhibited charts and reported "Normal Haematological Standards" based on an extensive study of different age groups between the ages of four and thirty years. For three groups representing the ages of 4 to 7, 8 to 18, and 19 to 30 years he gave the means for the total leucocyte count for the respective groups as 10,400, 8,300 and 7,400; and in the same order the limits of the counts were given as 6,000 to 15,000, 4,500 to 13,500 and 4,500 to 11,000; these are the limits which include 95 per cent of the individual counts. They show the tendency of both maximal and average leucocyte count to fall as adult age is approached. Osgood's data, which

were generously submitted to us by him, have been compared with data which include the leucocyte counts on 500 supposedly normal children between the ages of 5 and 19 years. The latter data were secured during a survey of hookworm conducted by the Rockefeller Foundation and directed by Dr. A. E. Keller who kindly placed the findings at our disposal. Our comparison has been made in the form of the frequency distribution curves shown in figure 1. The two distributions are strikingly similar and their significance will be discussed in relation to similar distribution curves constructed for adults. Both series of counts on which the frequency curves for children have been constructed were made on venous oxalated blood, as recommended by Osgood and Haskins (1931) and the studies directed by Keller added the use of the rotor device of Bryan and Garrey (1934) for mixing cells and diluent.

Adults. The magnitude of leucocyte counts of normal adults, if taken without regard to time of day or physiological conditions which are known to cause variations, show a fairly wide range. Galambos (1912) reported a range from 3,500 to 12,500, Feinblatt (1923) from 6,200 to 15,600, Fairley (1923) from 4,500 to 15,000, Sabin, Cunningham, Doan and Kindwall (1925) from 4,200 to 13,680 and Osgood (1934) from 4,400 to 13,250. In a new series of 627 observations on 200 subjects under random physiological conditions Bryan, Chastain and Garrey (1935a) found limits from 2,700 to 14,000. Relatively few reports have been presented in such a way that one could determine the frequency with which a given count, within the normal range, would occur in a normal population. We have constructed cumulative frequency curves, or ogives (fig. 2) which represent the published data of a number of authors who have studied the total leucocyte count in normal adult individuals; the studies by Shaw (1927), by Schweizer (1933) and by Bryan, Chastain and Garrey (1935b) were made during rest in the early morning. In the studies of Galambos (1912), and E. E. Osgood (1934) samples of blood were taken at random during the day but those of Torday (1913) were made on fasting subjects in the early morning hours (9 to 10 a.m.). This type of frequency curve has been selected since the character of the curve is not fundamentally altered by differences in class interval groupings, which vary with different authors, and it is particularly well adapted to interpolation. Thus if it is desired to find the value of the leucocyte count below which 50 per cent of the observations lie (i.e., median) or below which 95 per cent of the observations lie (i.e., 95th percentile), these values may be

approximated from the results of any of the authors represented in figure 2. From this figure it is seen that values for the median vary from 5,600 (subjects at rest in the early morning, Bryan et al.) to 7,420 (random counts, Galambos); the 95th percentiles vary from 7,800 for subjects at rest in the early morning (Bryan et al.) to 11,000 for random counts (Galambos, Osgood).

If one takes the commonly accepted upper limits for the normal count as 10,000 cells—the figure which overlaps the low limit postulated for patbological leucocytosis—and considers the highest pitched curve in this group (Galambos), one finds that 89 per cent of the counts on

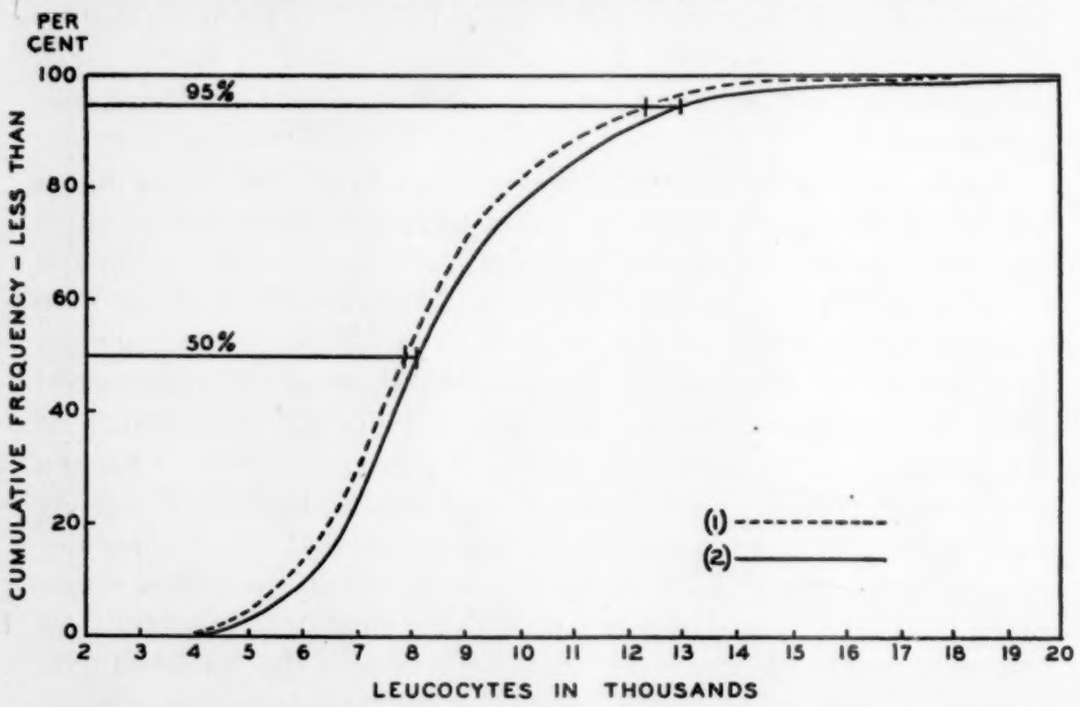

Fig. 1. Cumulative frequency distribution curves of leucocyte counts of school children. Curve 1, broken line, data furnished by Rockefeller Foundation Survey directed by Dr. A. E. Keller; curve 2, solid line, data by Dr. E. E. Osgood.

normal subjects are below this figure while 11 per cent are above it; however, only 5 per cent of these counts are above 11,000 and the chance that as high a count as this will occur in any individual under ordinary physiological conditions according to this curve is only one in twenty. On the other hand, if one takes the lowest pitched curve (Bryan et al.—rest in the early morning) one finds that the 95th percentile falls at 7,800, a figure only 800 cells higher than the limit found by Garrey and Butler (1929).

When one compares the frequency distribution curve for adults (fig. 2) with that of children as shown in figure 1, it is readily noted that

the whole curve for children is pitched at a higher level than that of adults; the median is 8,000 cells, i.e., 1,000 above that of the entire population of adults, while 20 per cent of children have a count above 10,000 cells.

Such distribution curves give no information relative to the leucocyte counts of the individuals comprising the population, since they include all the variations of all the individuals. As has been stated previously

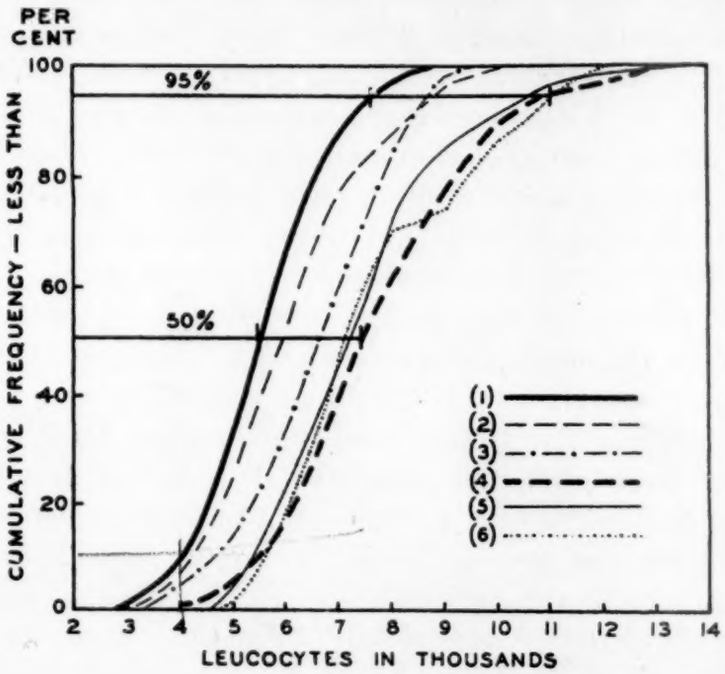

Fig. 2. Cumulative frequency distribution curves of leucocyte counts of adults. Curves are from data in published reports of the following authors:
(1) Bryan, Chastain and Garrey (1935b), heavy solid curve counts after one hour's recumbent rest in the morning.
(2) Shaw (1927), light broken line, morning rest.
(3) Torday (1913), dot and dash curve, morning counts on fasting subjects.
(4) Galambos (1912), heavy broken line, random counts throughout the day.
(5) Osgood (1934), light solid curve, random counts.
(6) Schweizer (1933), dotted line, counts during morning rest.

no individual shows a range as great as that of the whole population and a study of the literature indicates that the distribution curves of different individuals are relatively characteristic of individuals both as to the level at which they are pitched and as to the range (Doan and Zerfas, 1927; Smith and McDowell, 1929; Shaw, 1934). Thus one individual in our own series had a low count of 2,700 cells and over a period of three years' observation his random count has not exceeded 4,500 cells, while a maximum count after severe exercise was only 5,100 cells.

This individual would be readily distinguished in our series from another who for example had a minimal count of 6,120, a maximal random activity count of 11,200 in a series of 68 counts, with a mean of 8,244 cells; and in whom most strenuous standing running raised the count to 15,040 cells. This distinction has been emphasized by Miller (1914) and by Medlar (1929).

Data for the construction of distribution curves of individuals are very meager. Harvey and Hamilton (1934) have published ogives showing the distinguishing differences in the leucocyte counts of a normal and a pathological individual based on serial counts for 100 successive days. They discuss the statistical method of analysis of such data and the significance attributable to them. Shaw (1934) has given and discussed a distribution curve of daily counts made on a normal individual for one year. The observations of these investigators show that the total range is no greater than that of the same individual for a single day but is less than in a population and Shaw therefore concludes that the number of leucocytes is a "character of the individual." Shaw's experiments (1927) were conducted under controlled conditions which definitely decreased the variation, but the difference between the individual and population is striking; in a population of 116 normal subjects with a mean count of 5,987 the standard deviation was 1,460 and the coefficient of variation was 24.0, while the individual observed over a period of one year had a mean count of 5,770 cells with a standard deviation of only 772 and a coefficient of variation of only 13.37 (Shaw, 1934).

Daily variations. Fluctuations in the magnitude of the leucocyte count are mentioned throughout the older literature. Türk (1912) recorded low counts in the early morning hours; variations were designated as digestive leucocytoses, or they followed exercise (Zuntz and Schumberg, 1901). Independent of such possible determinants, recent work indicates that considerable variations may take place with marked rapidity (v. Liebenstein, 1924; Kobryner, 1924) and are ascribed by Hahn and Bramann (1925) to variations in velocity of blood flow in the superficial circulation. A new impetus was given to the study of variations in the leucocyte count by the appearance of a report and analytical review by Sabin, Cunningham, Doan and Kindwall (1925). These workers made total and differential leucocyte counts on a series of subjects at fifteen minute intervals, they reported a characteristic hourly rhythm and a definite rise in the afternoon counts which was independent of digestive processes. The fluctuations were restricted, in the main, to variations in the number of polymorphonuclear neu-

trophiles. Shaw (1927) found an afternoon rise due likewise to neutrophile increase, this increase being one of two high peaks or tides, the other occurring about or after midnight. Reinert (1891) had described two such daily maxima but they were thought due to digestive leucocytosis since they occurred approximately four hours after meals. Shaw failed to find rhythmic variations or the pronounced oscillations reported by Sabin and her colleagues, but noted minor fluctuations occurring without regularity or rhythm. Similar minor changes, but no rhythms, are also reported in this country by Smith and McDowell (1929), and by Jones, Stephens, Todd and Lawrence (1933). Recently Shaw (1933) has found night and day tides in owls as well as in pigeons which indicates their independence of diurnal or nocturnal habits. Shaw's afternoon tide in man amounted to 54 per cent in the total count and was independent of blood concentration or intake of food. To account for their observed variation Sabin and those working with her (1925) concluded from studies by the "supravital" staining method of Sabin (1923) that the hourly rhythm was due, in part at least, to the remittent production of new cells, whether from bone marrow or some other reservoir was not determined. They also postulate a corresponding death of old cells which is indicated by the appearance of periodic "showers" of non-motile cells. The significance of the non-motile cells in these observed leucocytic variations has been questioned on technical grounds by Smith and McDowell (1929) and by Jones, Stephens, Todd and Lawrence (1933) in the face of the careful and convincing controls made by Sabin et al. (1923 and 1925). Cooke and Ponder (1927) found that physiological leucocytosis in contrast to pathological leucocytosis is not accompanied by any departure from the normal leucocyte formula; there was no increase of the cells of their class I or class II and since bone marrow contains cells of class I, a few of class II, but none of class III, IV, or V they concluded that there was no addition to the circulating leucocytes from the bone marrow but the increase was due to the entry into the blood of a normal population of leucocytes "probably from the tissue spaces" (!). Shaw concurs in this explanation of the variation found by him. Smith and McDowell (1929) found variations occurring, not in hourly rhythms, but in "patterns" which they state are constant and characteristic for the individual. In a critical analysis of the studies on rhythms and oscillations which have been referred to above Ponder, Saslow and Schweizer (1931) used the periodogram analysis described by Whittaker and Robinson (1929) and concluded that only two of the six curves of Sabin et al. show even a moderately regular hourly oscillation, that none of Smith and Mc-

Dowell's curves show any recognizable periodicity, and since their own counts showed only very small oscillations ("residual variations"), they conclude that "periodic rhythms do not in general exist."

Zirm and Bauermeister (1933) have in a large measure confirmed Shaw's description of daily tides due to an increase in neutrophiles. They found the nocturnal rise less pronounced and of shorter duration and point out that subjects with relatively low leucocyte counts tend to show a smoother curve of variation; they found more marked variation and more pronounced afternoon tides when the count was high, which is in accord with our own findings (Bryan, Chastain and Garrey, unpublished); in fact we have found individuals with a marked afternoon increase both in level and range, while many subjects, usually with low counts, failed to show any such change. With these distinctions, the finding is in agreement with Ponder, Saslow and Schweizer (1931) who state that the afternoon rise "was an inconstant finding" in their series though it did occur in some subjects. They point out that there are similar exceptions in the results of Shaw and of Sabin et al., although the afternoon increase has been reported by a number of other investigators (Japha, 1900; Ellermann and Erlandsen, 1911; Mauriac and Cabouat, 1921; Naegeli, 1931; Arneth, 1920; Türk, 1912; Jørgensen, 1917; Sabin, Cunningham, Doan and Kindwall, 1925; Fairley, 1923; Shaw, 1927; Garrey and Butler, 1929; Zirm and Bauermeister, 1933).

That astonishingly large and quite abrupt variations in the leucocyte count sometime occur in normal individuals in spite of endeavors to eliminate evident excitants (v. Liebenstein, 1924) must be the experience of all workers in this field. The magnitude of the variations varies with the conditions of the experiment and with the technique used; furthermore the extent and frequency of the variation varies with the individual as stated by Zirm and Bauermeister (1933) who have described a high count with marked variations, 8,000 to 16,700 cells, in a vasolabile, highly emotional, convalescent girl. These authors report variations due to the intervention of other physiological states which may be given interpretations similar to those previously considered by Garrey and Butler (1929a, b). If one substitutes the term oscillations for rhythms and admits the advent of irregular and sometimes marked variations the apparent disagreements in the literature are of minor importance.

The basal norm. Naegeli (1931) emphasizes the fact that all leucocyte counts should be made in the early morning under conditions of rest and states that a great majority of such counts fall between 5,000

and 6,000 leucocytes per cubic millimeter of blood and Arneth (1920) had made a similar observation.

Correlating the leucocyte count with the facts that metabolic rate, cardiac rate and blood pressure are stabilized at their lowest level under basal conditions of complete rest in the early morning, Garrey and Butler (1929) refer to the leucocyte count as "basal" when made under similar conditions, conceived by them to eliminate so far as possible the physiological causes of variation. A great majority of their counts, made after an hour of rest, fall within the range of 5,000 to 7,000 cells, although a few were higher. An approximation to this basal range was obtained by an hour of rest at any time of the day and was independent of food intake. A count above the 7,000 level was interpreted by them as evidence of physiological unrest or of a condition merging toward a pathological state, although there are certainly exceptions and limitations to this view. The generalization that the count is lowest in the morning hours under the condition of rest and that it varies least under these conditions is firmly established by the work of many other investigators (Türk, 1912; Jørgensen, 1917; Glaser and Buschman, 1923; Stetson, 1927; Martin, 1932a; Raisky, 1932; Zirm and Bauermeister, 1933; Kaminsky, 1935). It has been shown by Stetson (1927) that the count of a given individual under conditions of minimal metabolism varies from day to day as much as 30 per cent and in the six cases studied he found a maximal count as high as 9,500 cells and slight variations from day to day were found by Bryan, Chastain and Garrey (1935b). Shaw's study (1927) of 116 subjects in morning rest showed an average leucocyte count of 5,987, a minimum of 3,200 and a maximum of 9,650; the standard deviation of these counts was $\pm$1,460 cells and the coefficient of variation was 24.0. A re-survey of this subject by Bryan, Chastain and Garrey (1935b) includes 300 counts on 75 healthy male students under basal conditions in the early morning. The average leucocyte count was 5,618, the minimum was 2,700 and the maximum was 8,600; the standard deviation was $\pm$1,205 and the coefficient of variation was 21.5. Cumulative distribution curves of these counts and Shaw's appear in figure 2; there is a close correspondence in these curves, they show that 78 per cent of Shaw's figures and 88 per cent of those of the Vanderbilt workers fall below the upper limit, 7,000 cells, set by Garrey and Butler. A very different distribution is reported by Schweizer (1933) in a study of fifty subjects; her frequencies do not agree with those of Garrey and Butler as her range extends from 4,700 to 11,500 so that her frequencies closely approximate those shown in the curves for blood samples taken

under all physiological conditions (Galambos; Osgood). Analysis shows that 26 per cent of her counts are above the limit of those found by Bryan, Chastain and Garrey, while 16 per cent are above the highest counts recorded by Shaw (1927) or Stetson (1927); in fact Schweizer's report stands alone in these high values although Egoroff (1924) reports a basal count average on 387 Russian military students to be 7,120. It may be that the limits set by Arneth, Naegeli, and by Garrey and Butler will be found too low and it seems quite likely that the frequencies found by Shaw or by Bryan, Chastain and Garrey will closely approximate the true status of the basal count, although further studies on a more varied population may alter this conclusion (cf. Bryan et al., 1935b). Investigations by the latter group of workers have brought out the fact that after an hour of recumbent rest in the afternoon 90 per cent of the leucocyte counts fall within the *limits* of basal counts in the early morning; in a few cases, however, the counts are as high as 12,000, and even 14,000. The zone of fluctuation of resting counts in the afternoon then is clearly wider by the extension of its upper limit (cf. also Mauriac and Cabouat, 1921) and it is suggested that if the term basal count is to be used it must be restricted to resting morning counts. In no sense should the term connote a fixed leucocytic level for all individuals.

Posture. Bürker (1928) states that there is a higher count in the erect than in the recumbent posture and Garrey and Butler (1929a) reported similar findings on subjects upon standing after an hour of rest. These reports are out of line with others, thus Hasselbalch and Heyerdahl (1908) and v. Liebenstein (1924) report inconstant results and the latter author feels that postural changes do not alter the course of a continual physiological variation in the leucocyte count. Jørgensen (1917, 1920) states that lying down raises the count sometimes as much as 100 per cent above the standing count and Jacobsen (1926) confirms Jørgensen's findings but with less pronounced changes. Ellermann and Erlandsen (1911) made short interval counts following abrupt changes in posture; they state that without exception, but with individual variations in time relations, the assumption of the erect posture causes a temporary fall in the leucocyte count coming on in 15 to 45 seconds and lasting 45 to 90 seconds, the converse effect appeared in the change from standing to lying posture. Jones, Stephens, Todd and Lawrence (1933) found no significant variation with change of posture but indications of a slight transient drop immediately upon standing in 49 out of 65 subjects, thus confirming, in part, the work of Ellermann and Erlandsen. A reinvestigation has been made by Bryan,

Chastain and Garrey (unpublished) using a series of short interval counts; they also found a consistent slight temporary drop upon standing after an hour of rest but counts after five minutes were variable and apparently dependent upon the specific reactions of the individual subjects. Cheng (1930) reports differences in the leucocyte counts on rabbits, dependent upon the "stomach up" or "back up" position, but Schweizer (1932) believes that they are independent of the postural factor.

Random activity. It is established that vigorous exercise increases the leucocyte count of the circulating blood (cf. section on exercise); and that the number of cells is greater as the severity of the exercise increases (Raisky, 1932; Martin, 1932; Chastain, 1935) but it has been denied that "random activity" of ordinary routine causes any rise above the resting level (Shaw, 1927; Ponder, Saslow and Schweizer, 1931; Schweizer, 1933; Jones, Stephens, Todd and Lawrence, 1933). The counts reported by Garrey and Butler showed a distinctly higher leucocyte count for random activity subjects above that on the same individuals under basal conditions. More recently this question has been subjected to specifically controlled investigation by Bryan, Chastain and Garrey (1935b) whose results and analysis indicate that while the reaction of a single individual cannot be predicted, nevertheless, by arranging the subjects in class interval groups according to the initial cell count taken during activity, using 1,000 cells as the group interval, the results show that in all groups above the *6,000 to 7,000* group, there is a definite and significant tendency for the mean leucocyte count to be lower after one hour of rest than during the preceding random activity. This may explain some of the discrepancies referred to above, since Garrey and Butler's activity counts were all above 8,000 while none of the subjects of Ponder, Saslow and Schweizer (1931) were above 6,000 and the differences observed by them as well as those observed by Bryan, Chastain and Garrey in the low class interval groups were not statistically significant.

Exercise. That the leucocyte count is lowest under the conditions of minimal activity has been well established (cf. supra) and that muscular activity causes an increase has long been known (Schulz, 1893); thus Zuntz and Schumberg (1901) found that soldiers after a long march with a considerable load of equipment developed a leucocytosis averaging 43 per cent increase. Literature dealing with this topic is reviewed by Grawitz (1911) and other references are to be found in the later articles referred to in this section.

The effects of strenuous and sustained effort are well illustrated by

studies on marathon runners, who run approximately 26¼ miles within two and a half or three hours. A study of four such runners has been reported by Cabot, Blake and Hubbard (1901) and by Larrabee (1902). Larrabee's counts show that all the runners had a marked leucocytosis in blood samples taken within five minutes of the end of the race. The rise in total leucocytes is illustrated by the change from 3,700 to 20,800 in one instance and from 8,200 to 22,200 in another. The differential counts showed that the increase at this time was chiefly in the polymorphonuclear cells which represented 84.4 per cent to 91.4 per cent of the total leucocyte count. Larrabee, Tileston and Emerson (1903) add to the other observations of Boston marathon runners; they found leucocytosis in all, varying from 14,200 to 27,700, still high two hours after the race. Isaacs and Gordon (1924) found in seventeen marathon runners both an absolute and relative increase in the polymorphonuclear cells without evidence of newly formed or immature cells. Larrabee likewise had found few new cells, but Egoroff (1924) reports many new cells in the blood of Russian marathon runners who showed the same absolute and relative increase in the neutrophiles. The leucocytosis established by this sustained effort did not subside to the normal count after twenty-four hours.

The above mentioned American workers think the increased cell count is due to a redistribution of cells in the vascular bed while Egoroff thinks that the added neutrophiles come from the blood forming organs and are the result of a myogenic intoxication in the sense that it is caused by fatigue products (Grawitz, 1910). Such toxic effects are referred by Gaisböck (1929) specifically to the outpouring of lactic acid, but Edwards and Wood (1932) produced a threefold increase in the leucocytes of a dog without any detectable increase of the lactic acid of the blood. Calculation from Egoroff's data show that less than one-third of the cellular increment could be due to new cells and that two-thirds are merely mobilized from other sites of sequestration into the general circulation, which is in line with the fact that Naegeli (1931) takes exception to the term myogenic leucocytosis owing to the many organs involved and the numerous mechanisms invoked. Many forms of muscular exercise cause leucocytosis but do not involve an accumulation or toxic action of muscular metabolities; none the less the persistence of the leucocytosis after exhaustion is significant since in shorter, less exhausting races the leucocytosis disappears much sooner.

The leucocytes of the blood of football players (Edwards and Wood, 1932) showed an increase in the leucocytes between 200 per cent and

300 per cent, with counts as high as 27,000 which is quite as high as after marathon runs although the players were in action only eight of the sixty minutes of play; these investigators found a progressive increase in the absolute count throughout the game, an increase involving chiefly the neutrophiles. They found no appreciable change in the Schilling-Torgan band forms.

Short bouts of severe exercise caused marked leucocytosis up to 100 per cent or more, e.g., heavy gymnastics (Willebrand, 1903; Hawk, 1903). The participants in a great variety of short lasting athletic contests including a two mile run were studied by Hawk who found that all showed an increase in the total leucocyte count, averaging 43 per cent, with maxima of 97.8 per cent for the 220 yard dash and 104.4 per cent for water polo. The absolute maximum leucocyte count was 18,300 cells which is much lower than that produced by the prolonged strain of marathon runs. M. Schenk (1920) reported leucocytosis in ski-runners who showed counts quite comparable to those found by Hawk in corresponding athletic contests. It appears that a lymphocytosis as well as a neutrophilia occurs quite generally in these short strenuous bouts of exercise and is noted immediately after they are over. The similar lymphocytic increase in long lasting exercise apparently subsides during the exercise (Egoroff, 1924) and leaves the predominant neutrophilia (Weindrach, 1929). The lymphocytosis has been reported by Grawitz (1910), M. Schenk (1920), Naegeli (in rowers, 1931), Lieberow (1914), Ernst and Herxheimer (1924) and by Waldmann (1923). Garrey and Butler (1929) noted a leucocytosis of 35,000 immediately after a quarter mile run coupled with rage due to a foul; the blood in this case showed 55 per cent of lymphocytes. The most probable explanation of the lymphocytosis is referable to the increased lymph flow caused by exercise as demonstrated by Rous (1908).

The leucocyte count after a run of eight-tenths of a mile was followed by Schneider and Havens (1915) who found that the leucocytosis receded to the normal level in one-half an hour; the count in the case of Garrey and Butler, just mentioned, dropped to 13,000 within forty-five minutes. That the degree of leucocytosis increases progressively with the severity of work done upon a bicycle ergometer has been shown by Raisky (1932). His figures for differential counts indicate increases in both neutrophiles and lymphocytes in excess of the hemoglobin increase. Owing to the fact that leucocytic increase can take place after the maximal increase in red cell count, Raisky rules the spleen out of the picture as a significant source of the leucocytes. Similarly

Camp (1927) states that the spleen has little to do with leucocytosis of exercise and Garrey and Butler got activity leucocytosis after splenectomy, indicating other more significant sources of the cellular increase (cf. section on adrenalin). For literature in which the spleen is considered a significant source of the leucocytosis of exercise consult Grounelle and Warter (1930). Raisky's experiments quite definitely rule out concentration of the blood as a factor in the leucocytosis of exercise and your reviewers have calculated from loss of weight during exercise and from the increase in hemoglobin (even assuming it were due to blood concentration, which of course it is not) that such concentration could not account for more than one-twelfth of the leucocytic increase.

The rapidity with which the leucocytic shifts may occur is surprising. These have been pointed out by Garrey and Butler (1929) who found a high count in one individual of 22,000 cells after a dash of 100 yards and of 35,000 after a quarter mile run lasting less than eleven seconds in the one case and one minute in the other; the samples were taken as rapidly as possible after the conclusion of the race. Similarly they found that twenty knee bends raised the leucocyte count to 12,000 or 13,000 within two minutes with a return to normal within fifteen minutes when the subject was recumbent. Leucocytic increases of 19 per cent to 43 per cent produced by simply raising a weight are reported by Ellermann and Erlandsen (1911) to have subsided within two or three minutes; Bergman (1922) produced leucocytosis by raising the leg 30 cm. at a rate of 45 per minute, the increase showed within two to four minutes; Gaisböck (1929) reports that marked increases take place within a few seconds and Walterhöfer (1921) records similar rapid fluctuations. In the Vanderbilt Physiological laboratory L. L. Chastain (1935) has studied these quick responses. He has selected standing running as a standard exercise and found that a bout lasting thirty seconds caused a rise of as much as two thousand cells within the few seconds necessary to take the sample at the end of the bout. The count continued to rise for one to five minutes, sometimes longer, depending upon the vigor of the exercise and then receded more gradually. With the milder grades of exercise Chastain noted a 50 per cent recovery in less than five minutes which was complete after fifteen minutes of rest. More vigorous running (more rapid stepping) prolonged the rise and the recovery was delayed for a half hour. By grading the rate of stepping and the interval between bouts it was possible to obtain a 20 per cent increase due to each bout which was followed by a fall to the initial count and this sequence could be repeated

every five to seven minutes as frequently as desired. Similarly by stepping up the severity of the exercise and repeating it before the return to the norm it was possible to sum the effect of succeeding bouts and thereby to reach a degree of leucocytosis which equalled the maximum obtainable by that form of exercise. These reactions are significant for the formulation of the mechanism responsible for physiological leucocytoses as will be pointed out in a subsequent portion of this review.

Training. This phase of the relation of exercise to leucocyte variations is in too contradictory a state to permit any dogmatic conclusions. The subject has been reviewed by Thörner (1929) and briefly by Steinhaus (1933). M. Schenk (1920) gives figures which indicate that trained athletes show practically no leucocytosis after a 200 meter run and even records a drop in three subjects due to the race. He states that training also decreases the tendency to lymphocytosis. Gaisböck (1929) indicates a lower count and less change due to exercise by well trained subjects than by the untrained whose count continued to rise for a longer period and was more sustained. That there is less distortion of the leucocytic formula in trained subjects has been stated by Egoroff (1924).

In our own experience one subject showing our lowest count, averaging around 3,000 cells and another with a resting count around 4,000 white cells, both wholly untrained, showed only minimal increases with the severest exercise; another subject was a large and powerful football player whose count averaged around 3,500 white cells, his count too was little affected even by a football game and in the past seven years of complete lapse from physical training there has been no change in his leucocyte picture. Individual characteristics seem to be the controlling factor; nevertheless, while no hard and fast statements are warranted, it does seem that the counts of trained subjects with comparable counts of the same magnitude are less variable than those of untrained subjects.

Epilepsy. Owing to the relationship of the convulsive seizures of certain neurological states to muscular work a note concerning the leucocyte picture is relevant. Capps' (1896) classical description of the blood picture in general paralysis showed instances of elevation of the leucocytes by a single convulsive seizure above the level which has since been described for the severest marathon run; he records one such leucocyte count of 37,000. An exhaustive study of the leucocytosis associated with convulsions, made by Burrows (1899), also reveals

instances of enormous leucocyte counts, particularly in epilepsy, resulting from a single convulsion while much higher counts resulted from a series of convulsions which in one case raised the leucocyte count to 43,500, evidently by the summing of the effects of the individual seizures. The neutrophilic cells predominated frequently making up 80 per cent to 94 per cent of the total leucocyte picture. All subsequent reports agree with this early work. In addition to the myogenic factor these pathological conditions are the results of a general neurological upheaval involving asphyxia (cf. Fraenckel and Hochstetter, 1910) and a perversion of circulatory, respiratory and endocrine functions.

A review of the literature dealing with the blood picture in epilepsy has been made by Patterson and Weingrow (1929); citations have also been made by Lennox and Cobb (1928) and by Naegeli (1931).

Adrenalin. Adrenalin, however injected, causes a marked leucocytosis (Naegeli, 1931, gives references); the number of both the lymphocytes and neutrophiles is increased and with great promptness. Benhamou (1933) reports a greater absolute increase in the neutrophiles than in the lymphocytes, with an abrupt drop in the latter at the end of one hour so that there is a persistence of neutrophilia (also Walterhöfer, 1921). The duration of the leucocytosis and the proportion of the two chief forms of cells evidently depend on the dosage, mode of administration and individual reactions; Walterhöfer (1921) and Gaisböck (1929) report that the condition is over in 30 to 40 minutes, while Benhamou (1933) shows a neutrophilia after two hours and Garrey and Butler also found a marked lymphocytosis six hours after a large dose administered subcutaneously. A diphasic effect, i.e., first a lymphocytosis then a neutrophilia, thought by v. Frey (1914; et al., 1921) to be characteristic, has not been regularly observed by other investigators, although it does occur sometimes (Hatiegen, 1917). It has been stated that the neutrophiles show a slight shift to the left by both Gaisböck and Walterhöfer, but Naegeli (1931) states that there is no such change in the neutrophilic formula and therefore no appearance of new polymorphic cells; he concludes that the increase in this form of cells is due exclusively to a redistribution and liberation of sequestered cells already present in other organs, e.g., liver and lungs, without any special involvement of neutrophil forming organs. Repeated administration of adrenalin causes a hyperplasia of the bone marrow (Walterhöfer, 1921) and its involvement has also been reported by Schön (1924). Fegler (1927) has reported that adrenalin induces

a leucocytosis followed by a leucopenia and states that the leucocytosis phase is associated with contraction of the vessels of the whole vascular system and that the leucopenia results from the retention of a greater quantity of leucocytes in the liver.

On the assumption that the spleen was in large measure involved in the regulation of the blood supply of lymphocytes, v. Frey (1914) advanced the view that the lymphocytic increase after adrenalin injection constituted a functional test of splenic contractility. It has since been established that the leucocyte reaction to adrenalin is too complicated to admit so simple an interpretation (Naegeli, 1931). Barcroft (1926) has concluded that as yet there is no evidence that contraction of the spleen adds to the number of leucocytes in the splenic vein and states that Harvey's theory (1906) of this mechanism is not substantiated (cf. Camp, 1927). The crucial disproof of v. Frey's test lies in the fact that the reaction with lymphocytosis after epinephrin takes place after removal of the spleen in dogs (Fegler, 1927) and in man (Yang, 1928; Gaisböck, 1929; Jung and Collet, 1930; Benhamou and Nouchy, 1931; Benhamou, 1933). It has been shown by Davis and Carlson (1909) and by Rous (1908) that lymph is the chief, if not the sole, source of blood lymphocytes and since it is established that there is a marked increase in thoracic lymph flow due to adrenalin (Yanagawa, 1916; Lamson and Roca, 1921; Haynes, 1932; Bambridge and Trevan, 1917; Meyer-Bisch, Günther and Bock, 1926; Chistoni, 1921) this may be looked upon as the chief contributory source of lymphocytes after adrenalin injection. Florey (cf. Barcroft, 1926, p. 847) found that stimulation of nerves to the lymph glands caused contraction of their smooth muscle with an enormous increase in the white cells of the lymph, a fact which Martin (1932a) confirmed and invoked as a likely contributory factor in the leucocytosis of exercise which is often accompanied by lymphocytic as well as the more usual neutrophilic increase. That there is an outpouring of adrenalin as a result of exercise has been restated by Martin (1932a) who considers this to be the cause of the contraction of the lymph glands. Hartmann and Jokl (1930) attributed the initial lymphocytosis of exercise to the outpouring of adrenalin through its action on the spleen; but lymphocytosis occurs after splenectomy so that the spleen is eliminated as a factor; thus Garrey and Butler, in unpublished observations, found that exercise after splenectomy still caused a leucocytosis in three dogs and in two men, on one of whom a differential count showed a major increase in neutrophiles with but a slight increase in the lymphocytes; the exercise

was quite moderate. In view of the fact that the liver is an accepted site of blood storage (Mautner and Pick, 1915; Grab, Jansen and Rein, 1931; Barcroft, 1934), and also of leucocytic sequestration (Seyderhelm and Oestrich, 1927; Fegler, 1927) this organ must be considered to be a potential source of leucocytes and the effects of adrenalin on its circulation must be kept in mind; general aspects of adrenalin circulatory effects have been studied by Lamson and his collaborators (1915–1921) and by Bauer, Dale, Paulson and Richards (1932) but the relation of the liver reactions to changes in blood leucocytes is still *terra incognita*.

Digestion. The literature dealing with the relation of digestion to the leucocyte count from the inception of the idea of a digestive leucocytosis by Nasse (1850) and Moleschott (1854) to 1923 has been voluminously reviewed by Arneth and Ostendorf (1923); other reviews are to be found in the monographs listed among the references while shorter reviews are to be found in articles by A. G. Mitchell (1915); Sabin, Cunningham, Doan and Kindwall (1925); Plitchet (1927); Shaw (1927); Cheng (1930) and Gyllenswärd (1930). In this earlier literature most of the daily variations in the leucocyte count were believed to be due to food intake which was conceived to cause a gradually developing leucocytosis within one to four hours lasting from one to several hours and slowly subsiding; and an initial fall preceding a rise of 20 per cent to 100 per cent has been reported (Japha, 1900). Careful perusal of the early authors reveals the fact that some subjects failed to show any such effects of food, and that some showed changes without any food intake. Shaw points out that the recent finding of daily variations, oscillations, rhythms or tides (cf. discussion supra) which takes place without reference to food intake, changes entirely the interpretation of the previous conflicting literature which was based on counts without controls as to time of day, conditions of activity or rest and individual characteristics of the subject (Doan and Zerfas, 1927). The trend of recent investigations, in each of which at least some of these conditions are controlled, is to deny the existence of a digestive leucocytosis. The afternoon rise in the leucocyte count occurs regardless of food intake (Japha, 1900; Sabin, Cunningham, Doan and Kindwall, 1925); the individual variations found by Smith and McDowell (1929) persisted independently of meals; "the day and night tides occur regardless of food" (Shaw, 1927; Zirm and Bauermeister, 1933; cf. *Daily Variations*). Martin (1932) found only normal ranges of counts in six normal subjects. The feeding of heavy meals of protein (or carbohydrate) to subjects kept in the condition of minimal metabolism did not cause the

slightest rise in the low basal count according to Garrey and Butler (1929a; 1932) and this has been corroborated by Raisky (1932). Lawrence, Stephens and Jones (1933) report that 9 out of 15 subjects showed a slight increase in the leucocyte count following the ingestion of food but it was not a constant reaction and examination of their data shows that the increase, when found, occurred at the hours of the normal daily tide described by Shaw, so that the conclusions are open to the same criticism which Shaw applies to the older observations. Arneth and Ostendorf (1923) were not certain that the moderate rise in the leucocytes, seen by them, averaging 35 per cent following a protein meal, could not be accounted for on the basis of quite independent and normal conditions. They found that the Arneth count showed no alteration in the distribution of the various types of leucocytes and rejected the idea that any new cells are introduced into the circulation as a result of digestion. We have found record of numerous instances of a decline of leucocytes following the ingestion of food, thus in nursing infants Gregor (1898) found a leucopenia following nursing in nearly 50 per cent of his subjects and A. G. Mitchell (1915) has observed a similar drop in the leucocyte count in a large majority of the infants investigated; this may be attributable to the rest or sleep which follows feeding, if so, the drop would have been particularly marked had it been preceded by the "schrei" leucocytosis (lymphocytosis) described by Hess and Seyderhelm (1916; cf. also Gallo, 1924). In view of the many physiological responses and readjustments associated with alimentation, to deny that any of them can cause changes in the leucocyte count would be unjustifiable. The question needs investigation from many angles especially bearing in mind the important relations of rest and independent diurnal variations (Shaw, 1927). While there may not be a digestive leucocytosis *per se* due to the absorption of ordinary food products or their digestive split products, certain ingesta can cause leucocytosis, e.g., alcohol is said to produce a leucocytosis within an hour after taking (J. Schloss, 1923; Palmieri, 1928) and overindulgence in alcoholic beverages is likewise reported to produce a (long lasting) leucocytosis (Medlar, 1931) whether as a direct effect of absorbed alcohol or secondary to gastro-intestinal irritation is not decided, although it is certain that any associated nausea or vomiting results in leucocytosis (cf. emotions). Glaser and Buschman (1923) report that mastication and swallowing constitute a stimulus to leucocytic increase and the phenomena associated with exaggerated intestinal movements has a similar effect as stated by Smith and McDowell

(1929) who observed one subject during a gastro-intestinal upset. In the series investigated by Bryan, Chastain and Garrey (unpublished) one subject while maintaining the recumbent resting position, developed strong gastric and intestinal movements thought at first to be hunger contractions, but later they became cramp-like and coincidently the leucocyte count rose from 7,500 to 14,000 within a period of two hours; a mild diarrhea developed six hours later. Mild transient diarrhea was accompanied by leucocytosis on three occasions noted by Garrey and Butler. Medlar (1931) also points to the high leucocyte count coincident with a non-infectious gastro-intestinal disorder. Mild "summer diarrhea" in infants noted on the pediatrics service of Vanderbilt Hospital was accompanied by a mild grade leucocytosis preceding a leucopenia when bacillary toxic symptoms supervened. Vagus stimulation (acute), with accompanying hyper-secretion, hyper-motility and vascular changes in the alimentary tract and viscera, is said to cause leucocytosis (Müller, 1926a, b, c; v. Hoff, 1928) but such results are questioned by Bürker (1928) as are those due to pilocarpine which causes a secretory, motor and circulatory effect similar to parasympathetic stimulation. Neurogenic relationships are suggested by experiments of Szabuniewicz (1928) who withheld food from dogs after habituating them to a fixed feeding time, he states that a leucocytosis develops at the feeding time—a conditioned reflex (!). Garrey and Butler (1932) demonstrated that hot and cold drinks and over distention of the stomach produced an immediate short lasting increase in the circulating leucocytes, which was confirmed by experiments on dogs. Walterhöfer (1921) also obtained a leucocytosis with hot but not cold drinks. Kmietowicz and Koskowski (1924) on the other hand report an increase in the flow of lymph from the thoracic duct and in some cases a marked increase in the leucocyte count following the introduction of cold water into the stomach. The oral administration of hydrochloric acid (4 per cent) to dogs is stated by Ciaccio (1922) to cause a leucocytosis in the absence of food and to this factor Viale and Di Leo Lira (1927) attribute a presumed digestive leucocytosis.

The increased lymph flow during the absorption period might be expected to result in a lymphocytosis; that it does not is apparently settled by Arneth and Ostendorf (1923). Appraisal of the effects of these accompaniments of digestion on the leucocyte count still leaves the possiblity of post prandial leucocytosis along with doubt of the existence of a true digestive leucocytosis. Possibly variable reports are only to be expected.

Altered dietary habits such as those existing in Europe during the World War, scarcity of fat and high vegetable content led to an altered leucocyte picture, to the war lymphocytosis described by Kleinenberger (1917), Bockelmann and Nassau (1918) and Laempe and Saupe (1920). Egoroff (1924) also mentions the same condition in Russians and the subsequent recovery of the normal blood picture. The condition was ascribed to a persistent thymus and general hyperplasia of lymphoid structures (Ferber, 1919).

Partial inanition apparently produces extremely variable results which have been reviewed by Morgulis (1923) and by Jackson (1929). Curschmann (1923) did not find either a leucocytosis or a leucopenia to be constant or characteristic. Stefko (1927) studied 30 individuals during the Russian famine and recognized two types of inanition, one with blood concentration but without change in either the total or differential counts, the other with hydremia and a definite lymphocytosis.

Starvation. Morgulis (1923) has analyzed the literature relating to starvation, and a brief review has been given by Emmel and Streicher (1929) and a comprehensive study from many aspects is given by Jackson (1929). The studies have included the leucocytic reactions in many animal forms as well as man, e.g., fish by Schaeffer (1925); frogs, Blumenthal (1904); guinea pigs, Suzuki (1925); rats, Emmel and Streicher (1929); rabbits, Källmark (1911), Okintschitz (1893), Argaud and Billard (1911) and Poletaew (1893); dogs, Rieder (1892), Ljubomudrov (1893) and Howe and Hawk (1912). In general most authors report a leucopenia chiefly due to the decrease in neutrophiles, some observed a lymphocytosis with a neutropenia and some few record an eosinophilia which is inconstant. Morgulis (1923) comments on the great infiltration of the tissues with leucocytes as a contributory factor in the leucopenia of the circulating blood, there is an aggregation of the leucocytes not only in the wall but in the lumen of the intestine where they die (Mingazzini, 1900). Similar conditions maintain in hibernation (Morgulis, 1923). In this connection attention is directed to the finding of Bunting and Huston (1921) that lymphocytes normally leave the blood stream, "find their way out onto the mucous membranes and in particular onto the intestinal" mucosa (Bunting, 1922). In a study of starvation of Succi made by Luciani (1889–1890) the leucocytes were found to be considerably decreased on the seventh day of the fast; in a later study on this same subject Tauszk (1894) found a progressive decrease in the total leucocytes throughout the fast. Similar results

were obtained by Lehman, Mueller, Munk, Senator and Zuntz (1893) who studied Cetti's blood. A progressive decrease in the leucocyte count during starvation of human beings has also been reported by Benedict (1907); the findings in differential studies of the leucocytes have been less uniform. Other studies on the blood picture in human beings during starvation have been made by Charteris (1907), Lichtwitz (1923), Curschmann (1923) and Beeuwkes (1926). Recently Egoroff (1935) has followed a subject for fifty days of complete starvation with moderate water intake and states that there is no significant change in the white blood cells. Examination of his data, however, shows an initial fall (largely of lymphocytes) from 7,000 cells to 3,700 in the first twelve days during which there was a progressively increasing acidosis, about the middle of the fast there was an unexplained leucocytosis of 11,340 cells with a subsequent drop and with oscillations between 5,300 and 9,000 toward the end of the fast. The eosinophiles did not increase as reported by others. There was a neutrophilic left shift in the Arneth count and Morgulis (1923) has suggested relative to similar findings that in addition to indicating that new cell formation continues this finding may mean that in starvation the cells fail of transformation into polymorphonuclear forms.

Climate. We have found no clear cut evidence that there are any climatic differences or seasonal variations in the blood leucocytes although one individual, observed for several months by Stetson (1927), showed an autumnal decrease in polymorphs with a relative increase in the lymphocytes as compared with other seasonal counts; no change in the total count was mentioned. In a very complete year's series of daily counts on a single individual Shaw (1934) found no seasonal variations either in the total count or in the different types of leucocytes, except a short lymphocytic phase which he attributed to the action of sunlight.

High altitudes are reported quite generally to produce a leucopenia and absolute lymphocytosis (Stäubli, 1910) although Ruppanner (1920) reports an acclimatization leucocytosis with increase in all forms of cells with a left shift in the neutrophiles, but this has not been confirmed so far as we can find. The lymphocytosis is apparently not an altitude effect *per se*, but is referable to the action of ultraviolet rays in the sunlight, after other initial effects (Baumann, 1920; Stammers, 1933). The effects of short wave radiation have been extensively reviewed by Laurens (1933).

Pregnancy. A review of the early literature from the inception of the idea of a leucocytosis of pregnancy and labor by Nasse (1839) and

by Moleschott (1854) was published by Dietrich (1911) who presents his own observations in which he followed individual cases through pregnancy. He concludes that there is a slight but unmistakable leucocytosis during pregnancy, at least in primiparae, the leucocyte count increasing in the later stages as term approaches. A somewhat later review has been prepared by Baer (1916) who also reports investigations which lead him to the same conclusions. Sieben (1915) as well as others have noted a rise in the leucocyte count during the weeks just before delivery. The time of this change is interesting in the light of the recent report of Burwell and collaborators to the Vanderbilt Medical Society (unpublished, 1935) that in the last four weeks of pregnancy there is a definite decrease in the previously elevated cardiac output, a suggestion of rather profound vascular readjustments at this time.

There is complete unanimity in the finding that the onset of labor pains and that labor itself is accompanied by a neutrophilic leucocytosis averaging 17,000 leucocytes which is greatest in primiparae and with severe and prolonged labor. The count may reach 34,000 (Maxwell and Yang, 1924). The leucocytosis usually continues to rise for a day after delivery and then recedes to normal within four or five days. Recrudescent leucocytoses may occur in cases of post partum pains. Dietrich's concept that parturition leucocytosis is the result of resorption of toxic substances into the mother's blood has little to recommend it. Three factors already considered in this review seem to be involved: 1, circulatory readjustments accompanying the uterine contractions and parturition; 2, a leucocytosis due to the accompanying general muscular activity (Fauvet, 1932), and 3, the emotional reactions to pain (cf. *Emotional leucocytosis*). The leucocytosis throughout pregnancy, when it exists, is a neutrophilia as is that of labor and the puerperium. Many state that there is some shift to the left (Arneth, 1905; Baer, 1916; Maxwell and Yang, 1924; Damblé, 1930), but Heyn (1924) found no deviation from the normal neutrophilic formula during pregnancy and Fauvet (1932) who controlled the work factor confirms the finding, although he found a left shift due to labor, similar to the condition which has occasionally been reported to take place in exercise.

Recent work indicates that the menstrual cycle does not cause any significant changes in the leucocytes of the blood (Medlar, 1929; Smith and McDowell, 1929); an earlier report by Dirks (1912) states that there was a slight lymphocytosis in about half of the subjects studied while a somewhat larger portion showed a slight eosinophilia.

Emotional leucocytosis and affective states. The section of this paper

dealing with distribution presents evidence that the capillaries of various organs are the site of sequestration of leucocytes and that these can be mobilized into the general circulation by circulatory changes thus causing leucocytosis (Becher, 1920); *a priori* it would appear therefore, that a variety of emotional and psychic states known to affect the circulatory mechanism, either directly or indirectly, would affect the leucocyte count. There is ample evidence that this is the case (F. Hoff, 1928). It was reported by Pohl in 1889 that the intense odors of fruit and aromatics caused leucocytosis; and that the thought of food, mastication and swallowing called forth a leucocytosis corresponding to Pavlov's psychic phase of digestion was stated by F. Glaser (1924); similarly Szabuniewicz (1928) has reported a conditioned feeding reflex in dogs which caused a "conditioned" leucocytosis. The lack of adequate experimental controls leaves much to be desired before accepting these conclusions. Nausea and vomiting have already been referred to as causes of leucocytosis (Garrey and Butler).

Cohnstein and Zuntz (1888) teased rabbits and reported a resulting leucocytosis although the associated muscular activity may have caused the condition since Nice and Katz (1934) report an emotional leucopenia in this animal, which they state could take place after splenectomy. On the contrary Menkin (1928) described for rabbits a relative monocytosis which he produced by excitement and prevented by the removal of the spleen and by sympathectomy. In dogs an emotional leucocytosis was reported by Mora, Amtman and Hoffman (1926), evidently neurogenous in origin since the difficulty of producing a leucocytosis of any type in dogs after sympathectomy has been shown by Butler and Garrey (1931).

Differences in the leucocyte reactions of phlegmatic and excitable individuals were described by Ellermann and Erlandsen (1911) and attributed to differences in circulatory (cardiac) reactions. Recently Kasanin (1934) has reviewed a large number of psychopathic cases in which he finds high leucocyte counts referable to "restlessness, tension and overactivity," thus confirming the conclusions of Bowman and Raymond (1929). A "brain-puncture leucocytosis" has been produced by Rosenow (1930), following puncture of restricted brain areas, e.g., thalamus and corpus striatum, in which cases he reported a marked neutrophilia. Mora, Amtman and Hoffman (loc. cit.) refer to a leucocytosis developing before operation in non-infectious cases—a condition they refer to fear and apprehension, and an identical reaction was noted by Garrey and Butler (1929b) in a highly excitable and vasolabile

case of hyperthyroidism, under basal conditions; in the latter case the mere suggestion of an operation caused a rise in the leucocyte count from 7,000 to 18,000 cells. The threat of a finger stab, or mere contact of a blunt instrument has been found by Bryan and his colleagues (1935a) to cause a marked vasomotor reaction and a finger stab itself has caused fainting which indicates an adequate emotional circulatory readjustment to account for leucocytic shifts.

Such reactions are of course referable to reflex or psychic effects and are similar in nature to the effects of a local dash of ether to the skin (Garrey and Butler, 1929b) or to local application of cold or heat to distant parts of the body. Even in narcotized dogs it is reported that breaking a bone or opening the peritoneal cavity or handling the viscera cause a leucocytosis (Schulz, 1893).

Ether anesthesia produces a leucocytosis and various causes have been held responsible, even a coincident acidosis has been suggested (Seyderhelm and Homann, 1923) but Garrey and Butler found that narcosis with barbital compounds produced no leucocytosis in dogs and since subsequent inhalation of ether did not induce a leucocytosis it is suggested that emotional and reflex reactions and possible struggle during the stage of excitement may have been factors to reckon with.

Pain has been noted as a source of leucocytosis. Garrey and Butler have reported a marked rise in the count due to the pain produced by stretching the sciatic nerve over a tumor mass by flexing the leg, also as the result of an attack of angina pectoris. An unofficial report from one of our large hospitals indicates a considerable increase in the leucocytes during certain attacks of renal colic, not associated with infection, although one negative result has been observed in the Vanderbilt Hospital. The leucocytosis of parturition has been associated with the onset and severity of the pain (Hahl, 1902; Birnbaum, 1905), and Zangemeister and Wagner (1902) also noted a leucocytosis due to the "after pain" in the puerperium. Fleishhacker (1926) reported the appearance of "Stabkerniger" forms due to lancinating pains in tabes dorsalis. Owing to its important surgical implications the leucocytosis in pain, without associated infection, needs careful reinvestigation under controlled conditions.

In a test of the apprehension preceding an examination Gaisböck (1929) found a leucocytosis in only one of six cases, but his control normal counts were taken in the afternoon when, as has already been pointed out, the count reaches its highest daily peak and since the test counts were made in the early morning at which time the count is

normally low it is obvious that the conclusion is open to question. Garrey and Butler refer to leucocytosis produced in physically basal subjects by ten minutes of confusing oral examination. Indications that psychic states have important relationship to the leucocytic picture are contained in the report of Lewitina et al. (1931–32) that a shift in the leucocytic formula, usually a left shift, followed a two hour examination. Goldberg and Lipskaia (1926) found an increase in leucocytes in psychic work which they referred to an increase in phosphate metabolism. The blood sugar of football substitutes, not participating in the game, as well as of spectators was reported by Cannon and Fiske (cf. Cannon, p. 74) to show an emotional increase, but Edwards, Richards and Dill (1932) failed to find this increase in similar subjects. Such differences in emotional reactions may account for the fact that Edwards and Wood (1932) found no emotional leucocytosis in football substitutes, although corresponding Harvard subjects have been reported to us to show an increase in the total leucocyte count to 10,000 or 15,000. The stages of excitement in subjects with general paralysis were reported by Capps (1896) to cause leucocytosis even in the absence of actual convulsions although Kasanin (1934) refers this effect chiefly to activity. Interesting observations in this connection have been made on morphine addicts by Light and Torrance (1929) who found that the withdrawal stage, when it was accompanied by a highly excitable state, was also characterized in 8 of the 10 cases so affected, by a high grade leucocytosis, which in one case reached 27,000 white cells.

Affective leucocytoses are most strikingly shown during hypnosis according to Wittkower's report (1929) to the effect that with hypnotic sleep there is a drop in the leucocyte count to a lower norm in five subjects but that suggested affective states such as joy, sorrow, rage or jealousy, caused leucocytosis, often quite marked, "appearing most strongly in the inhibitory affects without motor unloading."

Distribution and redistribution. In the last analysis the relative constancy of the leucocyte count in any given individual must depend upon the balance of the leucocyte formation and ultimate disappearance, whether by disintegration or emigration is immaterial. Simple calculation based upon the rapidity with which a given increase in the leucocyte count takes place, or upon the magnitude of the fluctuations and the frequency with which they may occur, throws doubt upon any explanation of the fluctuations based solely upon the formation and disintegration of the cells. Such calculations indicate a doubling of

the leucocyte count in a few seconds, and the possible prompt addition to the circulating blood of at least four or five times the number of leucocytes already present. So far as neutrophiles are concerned Bunting (1922) has stated that after birth their formation occurs only in the bone marrow, yet Drinker, Drinker and Kreutzman (1918) were unable to demonstrate any great release at least of red cells from the bone marrow by even drastic circulatory manipulations. Previous references show that many leucocytoses are unaccompanied by any left shift in the leucocytic formula which might indicate the formation of new cells, and where the shift does occur the new forms make but a small proportion of the increment. The indications are that cells already present in the vascular system appear, disappear and reappear in the circulating blood; a concatenation of events which can only take place if there are sites of sequestration and release of these cells. That leucocytes can, and do, accumulate in the capillaries of the various organs has been demonstrated under a variety of conditions—it is a normal phenomenon and there is ample evidence that cells can be released by circulatory readjustment although there is little unanimity of opinion concerning the underlying mechanism for these circulatory effects or their regulation by the vegetative nervous system (extensively reviewed by F. Hoff, 1928).

The aggregation of leucocytes in capillaries by a process of leucocytic skimming has been observed under the microscope by one of us (Garrey), also by C. E. King in our laboratory; it may be seen in some of the moving pictures demonstrated by Clark, Clark and Swenson (1933). Capillaries packed with leucocytes were described by Sandison (1931), the process of leucocyte skimming was described by him (1932) and microphotographs of the condition were published by Fischer-Wasels (1933). The pathological accumulation of leucocytes in the capillaries under conditions of slowed circulation is too well known to need any comment. The normal presence of large numbers of leucocytes immobilized in the capillaries of rabbits has been demonstrated histologically by Shigeru Momouye (1929); this condition occurs chiefly in the capillaries of the bone marrow, lungs and intestine. Nye and Barrs (1932) likewise state that "the large number of granular leucocytes present in the lungs, liver, and to a less extent, in the spleen can probably be explained by the immobilization within or in close proximity to the smaller blood vessels." The accumulation of leucocytes in internal organs was recognized by Hofmeister (1887) and Pohl (1889) and unequal distribution due to accumulation in definite parts of the

vascular system was stated by Goldscheider and Jacob (1894). Schulz (1893) concluded that it was not proven that leucocytosis means an absolute increase in the white cells but might be due simply to another distribution in the vascular system. Samples of true circulating systemic blood quite uniformly show an almost identical count (Shaw, 1927; Shigeru Momouye, 1929; Nye and Barrs, 1932; Bryan, Chastain and Garrey, 1935a). If, however, blood samples are obtained by puncture of the organ, widely divergent counts will be obtained, not only from different organs but from a given organ depending upon which drop is counted. It has long been recognized that the first drop even from an ear or finger stab may have a higher count than succeeding drops (Ellermann and Erlandsen, 1911; Krogh, 1929; Bryan, Chastain and Garrey, 1935a). It has been pointed out by Müller, Petersen and Hölscher (1930) that after a cut of the liver or spleen has been made the first drop may have a count of 14,000, subsequent drops 8,000, then 6,000 (cf. also Hahn, 1924; Hahn and Bramann, 1925). The first drops after subsequent cuts likewise show high counts and Müller et al. (1930) state that such a procedure must be followed in order to obtain their high values. In view of the fact that the counts of the succeeding drops progressively fall until the lowest count corresponds to that of systemic blood, as has been found by Bryan, Chastain and Garrey (1935a), and further that no count from an organ puncture which yields free flowing blood can be lower than that in the systemic vessels, it follows that the high count is that of cells sequestered in the organ and the lowest count represents that of systemic, circulating blood. These conditions have not been clearly recognized in reports on the distribution of the leucocytes and are, we feel, mainly responsible for the confusion and contradiction found in the literature of this subject. This, however, does not explain the differences in the count of venous blood obtained from veins in different parts of the body reported by Schweizer (1933).

The study of leucopenia produced by the injection of foreign protein (organ extract, etc.) has thrown light on the question of leucocyte distribution and sequestration in various organs. While some of these leucopenias may be due to the disintegration of leucocytes (Löwit, 1892), which is certainly the case when antileucocytic serum is the lytic agency (Lawrence, 1935), leucopenia may also be brought about by a progressive segregation from the blood into the various organs, thus Bruce (1894), Andrews (1910) and Webb (1924) all found leucocytes packed in the lungs during anaphylactoid shock. The trapping of

leucocytes in the liver and lungs was reported by Ewing (1895) following intravenous injection of bacteria. Similarly a predominant involvement of the liver and spleen has been noted by Bull (1915) and by Wells (1917). After nucleic acid injections Doan, Zerfas, Warren and Ames (1928) found a leucopenic phase due (solely) to accumulation of leucocytes in the spleen; when splenectomized, the rabbits showed a leucocytosis. Other aspects of the leucopenic states have been presented by Doan (1932) who discusses the literature. The underlying reason for the anaphylactoid segregation of cells in various organs is not determined; chemotropic (chemotactic) effects are suggested by Goldscheider and Jacob (1894) and by Wells (1917), while mechanical obstruction induced by endothelial swelling was considered to be the cause by Ewing (1895) and by Silverman (1904). In the normal individual the filtering and sequestering action of spleen, lungs and liver are well known, thus Seyderhelm and Oestreich (1927) injected congo-red stained washed leucocytes from empyema cases into the vessels of rabbits and dogs. When injected into the splenic artery or portal vein they were taken up by the liver and disappeared by solution there, but if injected into the ear vein they accumulated in the lungs. Müller and collaborators have brought forward voluminous evidence that leucocytes can shift their abode from the vascular bed of one group of organs to another (e.g., internal to peripheral and vice versa) through the influence of the vegetative nervous system on the vascular relationships. The specificity of the reciprocal relationships which Müller describes may however require considerable modification. Your reviewers consider that the existence of observed depots of leucocytes which can be rapidly flooded into the circulating blood and account for the prompt institution of physiological leucocytosis is established. The concept does not in any way militate against the idea that the alteration of the leucocytogenic functions of the bone marrow or other leucocyte forming loci can alter the blood count; this has been demonstrated for example by the production of leucocytosis through the action of penta-nucleatides (Doan, loc. cit.; Reznikoff, 1928–30) or contrariwise by the production of leucopenia (e.g., agranulocytosis) such as has been attributed to the action of amido-pyrine and similar compounds. On the whole, however, these reactions lack the promptness of the physiological reaction under consideration and therefore are conceived as having an entirely different connotation, although one should not overlook the promptness of the appearance of "showers" of non motile (dying) cells described by Sabin, Cunningham, Doan and Kind-

wall (1925) or their experimental production in rabbits by the injection of sodium citrate by Beard and Beard (1927).

The idea that leucocytes are very short lived and that their intravascular life is limited to hours or three or four days (Weiskotten, 1930) is undergoing modification. Concerning the longevity of the lymphocytes little can be said with certainty (Bunting, 1922). Minot and Isaacs (1925) transfused blood containing a high lymphocyte concentration (89,000 cells) into the normal blood stream, the excess of cells disappeared without reaction in two and a half hours, their fate was undetermined. The observations of Bunting (1922) that the lymphocytes disappear in great numbers on mucous surfaces indicates a short sojourn in the blood stream but gives no indication of their longevity.

Frog's leucocytes have been kept alive over a year in a culture medium (Jolly, 1911). Mammalian neutrophiles apparently can live much longer than three or four days in cultures *in vitro*, thus Forti (1926) reports motile dog's leucocytes on the 17th day and M. R. Lewis (1925) found human leucocytes alive after 20 to 28 days. Indications of the age of neutrophiles are presented by their changes in the staining reaction (reviewed by Naegeli, 1931) and may also be judged by the progressive increase in the nuclear lobulation (Arneth, Schilling). This latter fact was capitalized by Ponder (1926) who by means of a single injection of thyroid extract into rabbits caused a large increase in his class I neutrophiles (new cells with undivided nucleus, cf. Cooke and Ponder, 1927), and by following changes in nuclear configuration he concluded that the duration of time which a polymorph spends in the circulation of rabbits is from two to three weeks. Ponder, Saslow and Schweizer (1931) point out that day to day studies on the development of one lobed cells into cells of two lobes, three lobes, etc. (cf. Ponder and Flint, 1926; Kennedy and Grover, 1927; Climenko, 1930) also supported this view. In concordance with these findings on lower forms we call attention to the fact that the blood of new born babes does not contain neutrophiles with polylobulation for 9 days or two weeks (Sanford, 1929b; Lucas, 1930; Blackfan, Baty and Diamond, 1930) thus indicating a correspondingly long time for ripening.

In culture studies M. R. Lewis (1925) reported that after several days there is a transformation of leucocytes into macrophages, epithelioid cells and giant cells (Lewis and Lewis, 1925–1926) and Hetherington has also noted similar transformations of the monocytes into epithelioid cells on the eighth to the eleventh day. Attention is called

to these facts on account of their bearing on the improbability that quickly developing and quickly subsiding physiological leucocytoses are the result of rapid death and correspondingly rapid development and maturation of new cells. The alterations in staining reactions which have been shown to be associated with degeneration (Seyderhelm, 1925; Mommsen, 1927) are not seen in circulating leucocytes and the morphological changes noted by v. Philipsborn, (1927) in cultured leucocytes as well as those recently demonstrated to be degenerative indices by Haden (1935) are not sufficiently demonstrable to have any significance for the mechanism of the rapid changes in the white cell count noted under physiological conditions. Finally in this connection may be mentioned the fact that abundant and rapid disintegration of leucocytes, as in leucemias, is accompanied by the presence of an excess of nucleic acid and purine bodies in the urine (Horbaczewski, 1887). The physiological leucocytoses are not marked by any such chemical indication of leucocytic disintegration.

The evidence as viewed by the authors of this review indicates that the rapid variations in the leucocyte count are possible only as a reaction in which leucocytes are released from sites of sequestration within capillaries in general—but more specifically in the liver and lungs, and to a lesser extent in other viscera. The rôle of the spleen is definitely restricted and that of the bone marrow relates to white cell formation and gradual continual replenishment rather than to quick mobilization. The increase of the lymph flow is the chief agency in the rapid increments of blood lymphocytes.

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THE SO-CALLED ORGANIZER AND THE PROBLEM OF ORGANIZATION IN AMPHIBIAN DEVELOPMENT

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In writing a review on the amphibian organizer, a mere enumeration of facts would lead to bewildering confusion rather than to a comprehensible conception. The writer felt that, in order to make sense out of apparently contradicting facts, the problem of "organizers," as it stands today, could not be discussed profitably except in its connection with the general and still largely obscure problem of embryonic organization. In the following article an attempt will be made to point out the bearing the two problems may have on each other; if, as a result, the theoretical discussion has expanded unduly, the blame must be attributed to the fact that the framework in which we try to shelter the facts is for the most part still a fragmentary, largely hypothetical scaffolding.

Ever since the introduction of experimental methods into embryology the strictly preformistic conception of development has been losing ground. We have come to realize that development means the bringing into existence of new characters, not the bringing into appearance of pre-existing latent characters; that the manifest increase in diversity and complexity which can be observed during development is real, not only apparent. Heterogeneity emerges in the developing system in two ways: by differentiation and organization.

Differentiation is the becoming different among each other of the various parts of the developing system; this term, taken in the broadest sense, means that the various parts (cytoplasmic fragments in the unsegmented egg; cells and their territories in the segmented egg) lose primordial features, common to them all, and acquire specialized features distinguishing them from each other, such features concerning cytological structure and function, metabolic rate, growth rate, and sensitization toward certain stimuli, e.g., hormones. Viewed from the community of parts, this is differentiation; viewed from the individual parts, it is specialization.

Organization expresses the fact that all these processes of differentiation or specialization take place not chaotically in haphazard distribution, but according to definite patterns in space and time. With regard to the whole, we call it organization; with regard to the parts,

localization. It presupposes the existence of a system of conditions limiting the degree of freedom of each individual part, until eventually the fate of each one has become unequivocally defined in accordance with the constructional and functional plan of the whole. Viewed from the system as a whole, this appears as control exerted over subordinated parts; viewed from the parts, as coördinated interaction and coöperation.

The fact that, generally speaking, each part of a developing system possesses more potentialities at an earlier stage than would be found in it at any later stage, has been brought out clearly by the work of experimental embryology. The evidence obtained by isolation experiments may be outlined as an example:

Amphibian blastulae, at a stage when the cells do not yet show any marked differentiation, were cut up into small fragments containing a number of cells each; thereby, the fragments are released from whatever specific influences and interrelationships might have been operating between them in the intact system. The fragments were reared in what appeared to be, if not an indifferent, at least an unspecific environment: in the cavities of a developed animal ("interplantation" into the orbit of the eye) (Dürken, 1926; Kusche, 1929; Bautzmann, 1929a) or into the abdominal cavity (Holtfreter, 1929a) or in a suitable medium outside the organism ("explantation" into salt solutions (Bautzmann, 1929c; Holtfreter, 1929b; Erdmann, 1931)). Careful work had been done previously to map out, by means of vital stains, the fate of the various areas of the blastula in normal undisturbed development (Vogt, 1925, 1929). By consulting these maps, it was possible to predict with fair accuracy to what sort of an organ any of the isolated fragments would have contributed, had it been left in connection with the rest of the germ, and any discrepancy arising from the isolation of the part could easily be detected. There were discrepancies: It was found that the actual differentiation of the pieces reared in organic media, in some cases, deviated considerably from their predicted normal fate. Cells, for instance, that normally would have formed part of the skin epidermis could differentiate into nerve cells, muscle cells, notochord cells, cartilage cells, etc., more or less chaotically (Kusche, 1929; Bautzmann, 1929a). We may disregard, for the moment, the factors that may have caused the cells to deviate from their normal course in this or that direction. We merely want to emphasize the fact that, at the time of isolation, those cells did not yet have a definite irrevocable destination.

Not all the fragments show the same degree of indeterminacy: there

are certain areas the cells of which never give rise to anything but to what they would have produced in their normal course. In the amphibian egg, the later blastoporal region is such an area; after isolation only notochord and mesodermal differentiations can be obtained from it, in accordance with its normal fate (Kusche, Holtfreter). It is not necessary, however, to contrast the two types of fragments with each other. There is good evidence on hand to show that the rigid stereotypism of differentiation, as displayed by the blastoporal fragments, merely anticipates a state of affairs that, in a later stage of the embryo, will characterize all the cells, taken from whatever area. This is demonstrated by the fact that those areas which in the earlier stage were found to be still capable of a considerable variety of differentiations, when isolated at a somewhat later stage, invariably tend to develop in the one direction that would have been their fate in the normal germ, just as the blastoporal material did from the earlier stages on (Holtfreter, 1929; Mangold, 1933b).

The perseverance in a definite course of a part of the developing organism, regardless of whether or not the typical relations to its surrounding parts persist, has been called "self-differentiation" (Roux, 1912). Demonstrated by the methods of transplantation and explantation, it has, for a long time, assumed a central position in embryological experimentation and theory. This dominant position has been seriously challenged, however, by the experimental work of the last decades. Initiated mainly by the systematic work of Spemann and Harrison, continued experimentation revealed more and more examples where self-differentiation in amphibia was unmasked as due not to a primordial capacity of the particular part in question, but to an acquired capacity, derived from specific influences, directions and limitations, to which the part had been subjected during its previous ontogenetic history. Materials that in normal development would form the eye will go very far toward producing an eye, even after being isolated from the rest of the embryo (Filatow, 1926), and we would call this self-differentiation; but they will do so only provided they have been isolated at a sufficiently late stage, while at an earlier period the same materials will be found in the state of indeterminacy described above.

Recognizing that, in these and many similar cases, the ability for self-differentiation has been acquired only gradually, one naturally becomes suspicious also of those manifestations of self-differentiation where the direct evidence, that it might be an acquired and not a primordial capacity, has not yet been furnished, as, for instance, in the

blastoporal region of the amphibian germ. Differentiations that seem to be irrevocably determined in particular regions of an egg, even as early as at fertilization, must not necessarily be assumed to have been so at still earlier stages, during or before maturation. But even if a general solution covering all the examples of so-called self-differentiation should not be found, self-differentiation, in any case, has ceased to play the dominant and universal rôle as *the* principle of development for which it had been proclaimed. This brings into the foreground the phenomena which are not pure self-differentiation.

All that is not self-differentiation was designated as "dependent differentiation" by Roux (1912). Using his terms, development could be described grossly as a gradual transformation of dependent differentiations into self-differentiations. To indicate this general trend of development, the terms seem satisfactory; their explanatory value is, of course, nil. The mere assertion that there is such a thing as dependent differentiation is meaningless, as long as one does not state of what the dependence consists. It has been the chief task of experimental embryology to define, with greater precision, the sort of dependence involved in "dependent" differentiation.

Let us turn back to those egg regions which, at the beginning of gastrulation, proved to be still in a stage of relative indeterminacy. These same fragments, at a slightly later stage, for instance in the neurula, would exhibit strict self-differentiating capacity in given directions. It is evident, therefore, that between the gastrula and the neurula stage something very essential must have happened to them, something that does not only permit them to continue to develop under the conditions of isolation, but that has even laid down definitely the way in which they shall develop. Within this period, the parts of the germ have passed from a stage of indeterminacy into one of definite determination, have received final assignments to specific topics. This process of transformation has been called *determination*. It does not express itself by changes visible immediately, or if so, we certainly have not yet learned to see them, nor do we have any other direct indicators for the process. So, we must content ourselves with drawing our inferences from the visible transformations, which, after a certain lag, eventually appear as the results of those earlier hidden activities. While there is abundant experimental evidence on hand to show that there are real events and effects behind what we call determination, we are still very much in the dark concerning their nature and operation.

There seem to be two distinctly different modes by which determination might be conceived to affect an undetermined material:

1. The material may itself contain a store of several different potentialities; then, determination would work simply by releasing or activating one of them and suppressing the rest.

2. The material may have no tendencies of its own and may be incapable of accomplishing anything on its own account; then, determination would mean active instruction, imposing organization upon such unorganized material.

Commonly, the problem has been stated as above, in the form of a strict alternative, and experiments, undertaken to contribute to its solution, were expected to decide in favor of either one or the other possibility exclusively. There was a marked tendency to single out one individual factor as responsible for determination, and, undoubtedly, this tendency was encouraged by some of the experimental facts (see below). Yet, if a really comprehensive view is taken, one realizes that the organizing effects, called determination, can hardly be accounted for by a single factor. Certainly no one has formulated this fact as clearly as Harrison (1933) in his symposium paper on determination. He sounded a warning against the tendency to attribute to the play of any single outstanding principle the conditions leading to determination and organization.

In my book on experimental embryology (1930), I found it necessary and expedient to distinguish clearly between at least five different component processes, coöperating in making up what then appears to be a unitary series of developmental events. Those component processes, which it is impossible to separate from each other except by suitable experimental methods, are: growth (cell proliferation), morphogenesis (cell movements), histogenesis (cell differentiation), organogenesis (emancipation of parts from the whole), and functional and correlative adaptation. This list can be supplemented by several other components (e.g., metabolic, endocrine, dietary changes) as outlined by Needham in his recent review "on the dissociability of the fundamental processes in ontogenesis" (1933). The fact is that, under appropriate experimental conditions, any one of these component processes can be obtained without the others, and that only under the particular conditions of normal development are they so well coördinated with, and superimposed upon, each other that a perfectly harmonious performance results. Since it is impossible to attribute to any of these components paramount importance, relegating the others to secondary

rank, the futility of all attempts to solve the problems of development through one single and all-including formula is self-evident.

Cautioned by these general considerations, one would hesitate to put the problem of determination in the form of such a rigid and strict alternative as we did above, opposing to each other the possibilities of mere activation and specific instruction. If, as apparently is the case, many types of processes are combined into what we call determination, is it not conceivable that one of them may be activation and another instruction, both co-existing, overlapping and coöperating? In fact, if an attempt is made to reconcile the experimental results, which otherwise would contradict each other, an affirmative answer to this question seems to be the reasonable solution.

Experiments, giving the impression as if determination were a one-sided act, affecting the indetermined material without reciprocity, imposing rather than evoking certain performances, are principally transplantation experiments on developing eggs, involving the exchange of materials from different regions of the egg. For instance: Ectodermal cells that normally would differentiate into part of the skin, were transplanted, during the gastrula stage or earlier, into the region where later the central nervous system will develop; it was found then that such transplants develop in accordance with the characters of their new location, i.e., they participate in the formation and differentiation of the neural system (Spemann, 1918, 1921). The same material, when transplanted into the mesoderm, was found to take part in the formation of myotomes, pronephros, or notochord, depending on which one of these formative processes was going on in its vicinity (Mangold, 1923; Bytinsky-Salz, 1929). While the isolation experiments reported above had shown that young material may not yet be bent into any definite formative direction, these experiments here seem to show that definite formative tendencies can be impressed upon such material by factors emanating from its immediate surroundings. These factors, inasmuch as they bring into play the differentiation of the material in a typical pattern and sequence, according to our introductory remarks deserve to be called "organizing" factors.

In order to test the various organizing activities present in various areas of an embryo, Holtfreter has performed an extensive series of experiments (1933b). Gastrula ectoderm, which had been proved to be not yet disposed of for any particular differentiation and to be still responsive to organizing exigencies, was grafted to various levels of embryos in the neurula or even later stages. Although, probably on

account of the differences in age and stage between host tissues and grafts, the latter did not generally participate in the formation of primary host organs, as in Mangold's (1923) experiments, but gave rise to more or less circumscript supernumerary products, the results of the two sets are essentially in agreement. That is, the supernumerary organs, differentiating from the grafts, bore approximately local character: head organs (eye, nose, ear, balancer) were produced on the head, ear vesicles and gills in the gill region, limbs in the limb region, pronephros and muscles in the anterior trunk region, and tails in the posterior trunk region, in all cases out of material of essentially identical origin.

Experiments of this sort have definitely established the fact that different regions in the embryo exert organizing influences upon the parts that happen to lie within their reach. Nothing in these results suggested that the materials utilized as grafts had been an active party in the ensuing performance and had not been moulded passively by their new locations.

Other experiments, however, emphasized a quite different view. The first indication that the relation between the determining factors and the affected material might be not quite so simple and one-sided can be discovered in Spemann's experiments of 1921 where the donors of the grafts and the receivers of the grafts belonged to different species. It was found that the organizing factors were capable of affecting material derived from a different species (heteroplastic grafts) as well as that of their own, even beyond family and order limits (e.g., from anuran to urodele; Geinitz, 1925; Bytinsky-Salz, 1929a, b; Schotté, 1930a, b). Spemann, working with two different species of the newt, Triton, had noted that an ectodermal flap taken from one species and grafted over the gill region of a host of the other species would be utilized there in the local formation of the host's gills, however at a rate which seemed to be characteristic of the donor species rather than of the host species (also Rotmann, 1931). This certainly indicated an active part on the side of the grafted tissue. Experiments with species belonging to more distant groups brought even more striking results: For donor and host two species were selected that differed to such an extent that one of them would possess organs which the other would lack. For instance, a pair of balancers is present on the head of some urodeles, but is absent in others. When indifferent ectoderm from the belly region of a balancered species was grafted to the head region of a non-balancered species, the transplanted material conformed,

as usual, with the tendencies of the new locality by differentiating true head characters; among these, however, appeared typical balancers, i.e., structures characteristic of the species that had furnished the material, and not of the one that had furnished the organizing factors (Mangold, 1931).

Still more illuminating are transplantations between anurans and urodeles, which differ considerably in the type of their head organs. The main distinguishing features are the suckers and the horny jaws in the anurans and the balancers and teeth in the urodeles used. Indifferent ectoderm, taken from the trunk of an anuran gastrula and grafted over the prospective mouth region of a urodele germ, was observed to participate in the formation of the head and the mouth of the host, but in doing so was seen to produce suckers and horny teeth, head structures of the donor species, strange to the host. In other words, the grafted material has been responsive to the local organizing urodele instructions, but has obeyed them in its own anuran way (Spemann and Schotté, 1932; Schotté, 1932).

Results like these have shifted the emphasis back to the responding material. They suggest the possibility that the local organizing factors may be very general and unspecific in the way they act, evoking simply a pattern of functions, the specific spatial arrangement and time sequence of which would be an inherent potentiality of the reacting tissues.

Advocates of this view could report a very clear case in their support:

Balinsky found that the transplantation of an ear vesicle under the flank of an embryo in the tail bud stage can result in the formation of an extra limb in the vicinity of the graft (1925, 1927a, 1933). Practically the same effect could be obtained, however, by using as graft some dead matter, such as celloidin (Balinsky, 1927b). Over the dead implant, a supernumerary limb tended to develop. It is apparent that the local alteration, irrespective of how it was achieved, had caused such an irritation of the surrounding tissues that new formative processes were initiated; the quality and organization of these processes, however, must have been entirely the concern of factors latent in the activated area, certainly not the work of the activating lump of celloidin. There is a close analogy between these experiments and some experiments of Child on hydroids in which it was demonstrated that local laceration of the body wall can cause the formation, in that location, of a new head, provided the irritation had been sufficiently strong (1932).

Still other facts could be enumerated that would be just as suggestive in outlining determination as being activation and release rather than instruction. But there are again certain other data which do not seem to fit into this scheme, either, inasmuch as they demonstrate that there is a specificity in organizing effects that practically amounts to instruction. We shall discuss these data below, but wish to mention them in this place, in order to make clear that the problem: activation or instruction, has not been solved in favor of either alternative.

Indeed, wavering between the two extremes has characterized the course of Experimental Embryology for the last fifteen years, and it is only with this situation in mind, that one can give a just account of the work dealing with the so-called "organizer." From the first description of the activities of a so-called "organizer" in amphibia by Spemann and H. Mangold in 1924, an admirably straight course has led to some fascinating results obtained recently by a number of workers in the field, suggesting that the principle to which the "organizer" owes its effects may be a well defined chemical substance.

When discussing above the experiments testing the self-differentiating capacities of isolated fragments of the amphibian blastula or gastrula, we mentioned the fact that not all parts of an egg at a given stage are found in the same condition, and that there is especially one area which is always found to be ahead of the others in its determination, namely, the sector of the blastula which during gastrulation is invaginated around the lips of the blastopore into the interior and there forms the ento-mesoderm. If any of this material is isolated or transplanted into a host germ, it keeps on differentiating in its own way (Lewis, 1907). Even in the strangest location it displays attempts at invagination, and its cells undergo their typical differentiations into notochord, muscle, or pronephros. The inflexibility of this material contrasts with the plasticity which characterizes other regions at the same stage. It appears as if some conditions directing development into a definite course had been consolidated in this region first.

It was a discovery of utmost importance when Spemann and H. Mangold (1924) found that the early ability of this area to self-differentiate is associated with a marked capacity of exerting organizing effects on surrounding tissues. The first experiments consisted of transplanting fragments from the dorsal blastoporal lip of a gastrula into the less active ventral side of another germ. While the graft in its new location merely continued in its initiated movements and differentiations without regard to the altered conditions, the host materials in its vicinity

were conspicuously deviated from their normal manner of differentiation. What happened was that the host ectoderm over the graft formed a medullary plate, later closing into a neural tube, while the deeper tissues of the host joined the graft in producing the mesodermal parts of a new embryonic axis, containing notochord, myotomes, and pronephros. As a result, a secondary embryo of greater or lesser perfection arose, composed partly of grafted material and partly of host tissue, the two often being utilized side by side in the formation of the same organ.

It is obvious that in these remarkable cases the graft has brought with it capacities beyond its own self-differentiating powers: It spreads organizing influences, forcing upon accessible material differentiations that are very different from what this material would have normally produced; those influences are furthermore of such a kind as to make an orderly and typical formation result from the combined performances of the grafted and local materials. The district which was found to exhibit, under the conditions of such and similar experiments, those far reaching capacities of organizing supernumerary embryos, was called the "*organizer*" by Spemann. Generalizing the results, the idea was advanced that what had been observed under the experimental conditions was merely the manifestation of functions operating in normal development in essentially the same way. Consequently, to the "organizer" district was tentatively assigned the rôle of being the primordial center from which organization was dealt out to the rest of the germ.

There were experiments to support this assumption. It was known that isolated ventral halves of a divided newt's egg did not develop to any extent, whereas dorsal halves could produce normal embryos (Ruud and Spemann, 1922). According to the "organizer" conception, the failure of the ventral halves to develop could be explained by the fact that they did not share in the "organizer" district, situated dorsally. In line with this interpretation, it was observed that an isolated ventral half can develop up to a certain point after part of the "organizer" district had been transplanted into it (Bautzmann, 1927). Further evidence was furnished by observations on the origin of embryonic reduplications. When two newt's eggs in the two-cell stage are fused with each other by superimposing them so that the dumb-bell shaped pairs form a cross, giant embryos develop which are single or double or even triple (Mangold and Seidel, 1927). Just how many develop, seems to depend mainly on the way in which the "organizer"

districts of the two eggs had been distributed at the operation: when, in the composite germ, all the "organizer" material formed one continuous area, a single embryo developed; if, however, several patches of "organizer" material were present, separated from each other by material other than "organizer," multiple embryos arose, one with respect to each center. Similarly, in frog's eggs, inverted in the two-cell stage so that the yolk sinking down along the cleavage plane caused a separation of the "organizer" material into two portions, double gastrulation and the formation of twins may ensue (Penners and Schleip, 1928a, b; Wittmann, 1929).

The capacities of the organizer district can be traced back to very early stages of development (Bautzmann, 1926). The material making up the "organizer" can be recognized as such before cleavage begins in the so-called "gray crescent" of the amphibian egg. Despite its early presence in the egg, the "organizer" district does not, however, exert any noticeable effects until much later in development, and the earliest manifestation of its effects is generally considered to be its influence on gastrulation and on the formation of the medullary plate.

We mentioned above that, even after beginning of gastrulation, the various parts of the egg, with the exception of the material of the "organizer" itself, do not yet reveal any definite trend of differentiation. This situation changes quite materially during gastrulation, so that, toward the end of gastrulation, more and more parts exhibit definite self-differentiation capacities. From this we conclude that during gastrulation a definitive emancipation of partial processes from the whole takes place, as a result of which, at the end of gastrulation, the originally unitary system of the germ appears to be parcelled into a number of partial systems, each one endowed with a certain amount of autonomy. If this dealing out of the gross pattern of organization were to be credited to the activity of the "organizer," gastrulation should be expected to be the critical stage to test it. At this stage the "organizer" district, previously a sector in the surface of the blastula, is gradually invaginated around the blastopore and is spread beneath the ectoderm, progressing postero-anteriorly and constituting the ento-mesodermal roof of the archenteron. It furnishes to the overlying ectoderm a supporting substratum. While the invaginating layer transforms into the notochordal plate and mesoderm, the overlying ectoderm transforms into the medullary plate. The close contact between the medullary material and the invaginating "organizer" material directly beneath it suggested that the primary effect of the

"organizer" may consist of causing the overlying ectoderm to develop into a medullary plate (Spemann).

Several experiments supported this view. Lateral defects inflicted upon the underlying mesoderm entailed corresponding quantitative defects in the overlying medullary plate (Lehmann, 1926). Furthermore, the time at which the material of the later medullary plate proved to have acquired capacity of self-differentiation coincided with the time when the contact with the underlying "organizer" material was established (Marx, 1925). One could safely assume, then, that the invaginated layer of "organizer" material contained factors capable of inducing the overlying ectoderm to undergo neural differentiation. It had been claimed by Goerttler (1926, 1927) that the sector of ectoderm of the blastula destined for the later formation of the medullary plate could accomplish this formation also in the absence of underlying mesoderm, providing the necessary movements of the ectoderm were not disturbed. This contention, however, lost its convincingness when Holtfreter (1933a, d, e) showed that the experimental basis of Goerttler's thesis was inadequate. Thereupon, there was no more serious objection to the view that the first formation of the neural system was really due to influences of the "organizer."

A process of this sort, in which determination of one part of a developing system is brought about through decisive influences exerted by other parts, has been named "induction." Although inductive influences were originally thought of as specific, the term "induction" has been stretched in more recent years by several authors to such an extent that it has no longer such distinctive meaning and has become ambiguous. It now covers all sorts of influences, from mere unspecific activation, to the very specific organizing effects laying down typical patterns in space and time.

The assumption that the roof of the archenteron is instrumental in causing adjacent ectodermal areas to assume nervous characters has been verified by transplantation experiments: If a fragment of the roof of the archenteron is transplanted beneath indifferent belly ectoderm, the latter frequently produces accessory neural organs (Marx, 1925). While proving the existence of the effect, these results did not, however, decide about its specificity. Does the graft simply turn on in the overlying tissue a chain of reactions, the pattern of which is laid down by the reacting tissue itself, or does the graft intervene in a more specific way, by projecting into the reacting materials a pattern that they did not contain before, not even potentially?

The answer depends on whether or not the details of organization of the induced formation, such as orientation and quality, show any definite connection with corresponding features of the graft. It was found that they do show such a connection. Since the examples of this fact, though conclusive, are not very numerous, they must be pointed out with increased emphasis:

In some of the first series of "organizer" experiments it had been noted that the induced embryos did not necessarily show the same axis orientation as the host embryos. Some were oriented almost perpendicular to the host axis, and intermediate positions were observed also (Spemann, 1927). Cases of this sort indicate that the orientation of the secondary complex cannot be merely the work of activated intrinsic patterns of the host, because such patterns most likely would correspond in their orientation to the basic patterns of the host. It is reasonable, therefore, to conclude that the orientation of the secondary embryos has been the work of orienting influences of the graft, at least in certain cases.

Further experiments could trace not only the orientation, but also the quality of induced formations to specific influences, imposed by the graft on its surroundings. In order to test whether the patterns of the induced neural systems, were provided by the grafts or by the affected host tissues, fragments from various regions of the "organizer" were brought to act on host tissues of different regions. Head regions of the "organizer" could be supposed to lay down conditions specific for head development, while posterior regions of the "organizer" might contain specific prerequisites for trunk or tail organization. Accordingly, both anterior and posterior fragments of the "organizer" were confronted with either head or trunk ectoderm. The homologous combinations (head mesoderm in head region, and trunk mesoderm in trunk region) yielded corresponding fragments of secondary embryos, that is, anterior or posterior pieces, respectively. These results, of course, could not decide the issue. However, the same trunk ectoderm that over a trunk "organizer" would transform into trunk organs or into accessory tails gave rise, when confronted with a head "organizer," to typical head organs, brain, eye vesicles, nose, balancer (Spemann, 1931). Similarly, head ectoderm, when confronted with "trunk organizer" could produce trunk characters (Hall, 1933). In other words, the same reacting material behaved differently in the presence of different grafts, conforming more or less strictly with the character of the graft. Hence, the influence spreading from a grafted

"organizer" obviously consists of something more specific than mere activation.

Additional evidence for this fact has been brought forward by Mangold (1933a). He subdivided the invaginated roof of the archenteron into four parts, representing four different levels, and tested the organizing capacities of these fragments by transplanting them under indifferent ectoderm: anterior fragments were then found to induce head organs (brain, eye, nose, ear, balancer), posterior parts induced tails, and intermediate parts induced trunk organs (neural tube, pronephros, and muscle). Furthermore, it had been observed that the organizing capacity, present in the entomesoderm at gastrulation, persists therein during its differentiation into the notochord, subsiding only gradually (Bautzmann, 1928). Again, anterior fragments of this older "organizer" induced the formation of head characters, regardless of whether they were working on head or on trunk ectoderm (Bautzmann, 1929b).

Materials other than the original "organizer" district, which for some time have been under its influence, may acquire organizing capacities of their own. For instance, ordinary ectoderm which normally would not exhibit organizing effects is enabled to do so, after having been incorporated temporarily in the "organizer" region (Spemann and Geinitz, 1927). Similarly, a neural plate, after having received mesodermal substratum, shows itself in possession of organizing capacities that practically equal the ones that formerly appeared to be the privilege of the "organizer" itself (Mangold and Spemann, 1927). And again, in transplantation experiments the specificity of these organizing effects was brought out most convincingly, each regional fragment of the medullary plate leading to the formation in the surrounding host tissues of such organs as would normally be found at approximately the level from which the fragment had been taken (Mangold, 1929a, 1933a).

It is perfectly obvious that in this whole series of experiments the organizing factors contained in the grafts must have operated in a more specific fashion than by merely releasing a preformed sequence of processes dormant in the affected host tissues. Accordingly, the attempt to explain these effects as simple activation effects, as in Balinsky's limb inductions, must be abandoned.

There is one observation, however, which, recurring with a certain regularity in experiments of the type described, seems to complicate the interpretation: under certain circumstances, one observes that the affected host tissues give rise to a formation the quality of which is not

at all in accordance with the organizing tendencies of the grafted "organizer" fragment. Thus, secondary heads were frequently obtained in the head region not only after the transplantation of head "organizer" or of anterior parts of the notochord, but also after the transplantation of trunk "organizer" or of posterior notochord (Spemann, 1931). Therefore, as we see, accessory head structures may appear in the head district, no matter where the graft has come from and what its specific character may have been. In cases of this sort, it is obvious that the graft, while initiating the secondary formations, did not determine their character, this latter showing typical features of the affected host region.

In order to understand this situation, we must bear in mind that the host germ naturally possesses its own original system of organizing agents; the grafts, therefore, do not introduce into the host a monopoly for organization but simply bring in a supernumerary set of organizing agents. Whether, under these conditions, a given area of the host will receive its organizing instructions from the graft or from the host, will largely depend upon the mutual relationship of the two: If the tendencies of the graft coincide with the local organizing tendencies of the host, they may reinforce each other. If, however, the two tendencies conflict, the result will be determined by the prevailing one. Ascribing to a given graft a certain organizing power, it is evident that, the weaker the organizing agents of the host in a given area are, the better chance there is for the graft to supersede them and to produce organizing effects of its own. The various organizing agents of the host seem to be most effective in the areas where their respective activities are manifested in normal development: Experimental evidence shows that the factors tending to organize a lens (Spemann, 1912), or a limb (Harrison, 1918), or a balancer (Harrison, 1925), or an ear vesicle (Kaan, 1926) are most intense in the centers of the areas where normally the formation of lens, or limb, or balancer, or ear vesicle takes place. With increasing distance from these centers, the intensity of their respective organizing powers decreases. Accordingly, any grafted fragment possessing organizing tendencies of its own, but conflicting with the tendencies of the local factors, will succeed in imposing its own influences on the affected material only provided the location, where it is brought into play, is far enough away from the host centers.

It seems appropriate to introduce here into the discussion a concept which, owing to its empirical usefulness, has been widely adopted in experimental embryology. This is the concept of "fields" of organi-

zation. The term "field" has been created to emphasize that the pattern of the activities of the whole is not, and cannot be, arrived at by piecing together the individual activities of its constituent parts, but that, on the contrary, the activities of the parts are specifically directed and controlled by the conditions of the whole system.

In his first description of the "organizer" effects (1921) Spemann made the remark that it appeared as if "a field of organization" were produced by the "organizer." Although Spemann himself made no attempt at circumscribing or further evaluating the term, he undoubtedly was the first to suggest the field-like character of organizing effects in embryology. Independently and almost simultaneously Gurwitsch (1922, 1927) advanced a similar idea in a more elaborate form, offering specific examples of how "field" activities manifest themselves in the configuration of developing systems (also Anikin, 1929). Coming from a third direction, namely, from the experimental analysis of regeneration in amphibia, again the concept of "fields" of determination was arrived at (Weiss, 1923, 1925, 1926a), and after proving its value for the description of regeneration phenomena, has been generalized so as to include ontogenetic organization also (P. Weiss, 1926b, 1927, 1930). Ever since that time, the term has been accepted by numerous embryologists (e.g., Huxley and de Beer, 1934). De Beer (1927) and Child (1928) attempted to identify a "field" with a "system of gradients" in the sense of Child. It seems more appropriate, however, to put it the other way around and to consider a gradient as an index of a "field" vector.

Definitions of "fields" in terms of their properties have been attempted (P. Weiss, 1926a, b; 1927; Gurwitsch, 1930) without prejudicing the nature of the dynamic agents involved. The purpose of introducing the term into embryology has been to point at a certain fundamental reality in the dynamics of development, namely, at the fact that the dynamics controlling the whole system, being more than a mere sum of independent elementary events, have definite characteristic patterns of their own. To name these, means simply to make them accessible to intelligent approach, as objects of research that formerly, though recognized, have been largely neglected or even been barred from approach by unwarranted claims of their inapproachability. The emphasis, that there is such a thing as "field" activity, deserving attention and experimental attack, as well as the description of whatever of its properties can be deduced from its manifestations, is about all there is to be gained by the introduction of the term. It has proved

to be of descriptive, but not yet of ultimate explanatory value. It presents a problem rather than solves one.

If we try a cautious definition along these lines, we should arrive at about the following: A "field" is a system of *patterned conditions*, the pattern of which is not pieced together by individual contributions of independent constituents, but is the expression of the dynamic activity of the whole system, the whole pattern tending to retain its typical organization beyond changes involving its parts. The activities playing in the whole, or rather the conditions releasing those activities, e.g., potential differences, may they be of mechanical (tension) or electrical or chemical or whatever nature, are graded and distributed in some typical way, and this pattern of gradation and distribution tends to persist as such. The marked tendency of the system to persevere in its typical state is expressed by its capacity to return to this state (regulate) after disturbances. It is quite possible that eventually "fields" will turn out to be merely "systems" of specific configuration in space and of specific transformation in time (compare Köhler, 1927). In any case, they certainly seem to comply with the rules that hold for organismic systems in general (P. Weiss, 1924).

Summarizing, the "field," in our conception, is a pattern of conditions (if one wants to visualize it more concretely, one may tentatively think of tensions, potential differences, and the like), in a typical and self-sustaining configuration. The potentialities (e.g., potential differences) contained in the field, when released, yield actual morphogenetic and histogenetic effects partly directly, partly by being reacted to by responsive cells or nuclei. Their interactions necessarily distort the original field pattern to such an extent that there is to be expected neither a geometrical similarity between a field and its product, nor between products of identical fields acting under different conditions. Keeping this in mind will prevent gross misunderstandings.

In the terms of the "field" conception, the results of the experiments reported above can be summarized as follows:

A primary totipotent field has its center in the dorsal-animal area of the blastula and early gastrula; the ento-mesodermal material in this field represents the so-called "organizer." Under its activity the medullary field is established. During gastrulation, an emancipation of partial fields out of the original field takes place leading to a parcelling of the germ into areas with partial organizing capacities; from this emancipation emerge eye fields (Adelmann, 1929), ear fields (Kaan, 1926; Röhlich, 1929), gill fields (Ekman, 1922; Rotmann, 1931), limb

fields (Harrison, 1918; Balinsky, 1933), heart fields (Ekman, 1925; Copenhaver, 1926), etc. Possessing a field may enable an area to a certain degree of self-differentiation. Any such field affects materials within its reach in its own way; the eye field, for instance, by segregating the layers of the retina, aligning the sensory cells, inducing the overlying ectoderm to produce a lens, later a cornea, etc. (literature in Mangold, 1931b).

As the intensity of each field is greatest in its center and gradually decreases toward the periphery, the partial fields will establish their limits by virtue of their own mutual activities, each one extending its sphere of influence as far as the effects of the surrounding fields will permit. The boundary will become laid down as some sort of no-man's-land along the lines where the effects of two neighbouring fields balance each other. The fate of a cell or cell group will depend upon the character of the field within the domain of which it happens to lie. With field intensities fluctuating, the position and configuration of their boundaries must also vary. If the intensity of one partial field is diminished, part of its former ground will be occupied by the expanding neighbouring fields, and vice versa.

Now, with this scheme in mind, how can we interpret the grafting experiments? If inactive, but responsive, material is grafted within the reach of an active field, as is the case when indifferent ectoderm is grafted to various places in a host germ (Spemann, 1921; Mangold, 1923; Holtfreter, 1933b; see above p. 644), the outcome is clear: the material will be subjected to the specific influences irradiating from the local field and will behave and react accordingly, whereby, as the heteroplastic and xenoplastic experiments have shown (Mangold, 1931a; Spemann and Schotté, 1932), the possible reactions of the material are strictly confined to the formations and differentiations of its own species. In the reciprocal experiments, however, when active material, carrying with it some field of its own, is grafted into a host, the results cannot be uniform. In every case, as a result of the grafting, some sort of activation of the surrounding tissues is obtained, which initiates the formation of supernumerary structures. As for their quality, these formations, however, depend on the conditions of the field dominating the location. This may be the field of the graft, in case the location is removed so far from the host's most active centers that the field of the graft will encounter no considerable competition. If, however, the grafted field is not strong enough to supersede the host fields reaching over into the location, then these latter will seize the activated material and shape it.

As long as we are ignorant as to what determines the intensity of a field, it will be difficult to predict the outcome in any particular case. Possibly, in the case of grafts, the intensity of the field carried along by the graft is in a certain ratio to the size of the graft; at least such could be deduced from the fact that large grafts succeed much better with their organizing activities than do small ones (Mangold, 1928; Bytinsky-Salz, 1929b). But, as we shall see below, other explanations are also possible.

From the experiments of Spemann (1931) described above, where an accessory head was induced in the head region by trunk "organizers," one is led to infer that the head field had been stronger, even at some distance from its center, than the grafted trunk fields. But while this may account very well for the fact that the induction turned out to be a head, one must keep in mind that the head field alone would normally have concerned itself only with the formation of the primary head, without showing any attempt at organizing an extra head. Consequently, the graft, while not being responsible for the quality of the extra formation, must be credited with the fact that an extra formation was brought into existence at all. Thus, we come to the conclusion that the influences exerted by the grafts are of a double nature. First, they activate surrounding tissues in some way, perhaps simply by introducing a local disturbing factor into the otherwise well-equilibrated system of activities playing in the host germ; thereby, a first start is given to the tissues to produce something without possibly containing any definite directions as to what to produce, an absolutely unspecific start. Once started on their way, the surrounding tissues will begin to respond to whatever organizing field activity lays claim on them. This may be the activity of the host field extending over the locality, or it may be the activity of the grafted field, provided it is strong enough. And only in this latter case do we see the graft coming in with the second sort of activity, with specific organization, contrasting with its former unspecific activation.

By accepting this viewpoint, we introduce a strict distinction between two different forms of induction: in one case, the graft activates a field in the affected host region; in the other case, the graft, in addition to its activating rôle, furnishes itself the field and projects it into the affected tissues. The first case is realized in pure form whenever the graft lacks completely a field of its own; for instance, in Balinsky's experiment with celloidin as an inductor of a limb. This type has recently been called "evocation" by Needham, Waddington and Needham (1934). The second case is obtained whenever the graft possesses

an active field strong enough to supersede the activity of the host fields. Organization being field action, the inducing agents of the former type, which merely elicit field activity without displaying any themselves, do not deserve the name of "organizers."

The distinction is very important. Unless it is firmly borne in mind, it will be difficult to arrive at an intelligible account of the most startling course the "organizer" problem has recently taken, namely, its attempted analysis from the strictly chemical side.

It had been suggested that the "organizer" may owe its peculiar power to a chemical substance (Spemann and Mangold, 1924). In an attempt to test this point, one began to eliminate all factors, other than chemical, that at first might be suspected of playing a rôle in the phenomena of induction. Thus Marx (1931), for instance, demonstrated that "organizer" material, obtained from germs anesthetized by chloretone, could induce the formation of secondary neural tubes. Anesthesia evidently did not abolish the organizing capacities. Spemann (1931b) found that the "organizer" material could manifest inductive capacities after having been subjected to such violent treatment as crushing or mashing, destroying its histological texture. But as the crushed tissues exhibited an amazing capacity for rearranging and reconstituting themselves (Krämer, 1934), more conclusive experiments were needed to decide whether or not inductive effects were dependent upon the histological and vital integrity of the effective parts.

Therefore, work was begun by several experimenters along more or less similar lines, and in 1932 a joint statement by four authors, Bautzmann, Holtfreter, Spemann (for Wehmeier) and Mangold, reported induction effects obtained from "organizers," killed in various ways. The fact that some of these first-reported cases were rather doubtful, can be fully disregarded in view of the convincing results obtained by further experimentation. In Holtfreter's work (1933c, 1934a), the most comprehensive in this direction, the materials which were to be tested for their inductive capacities, were killed by heating at 60°C. (1933c), or at 145°C. (1934a), either dry or in salt solution, for about half an hour, or by boiling in water 1½ hours, or by freezing. After this treatment, the fragments were brought to act on normal responsive ectoderm in one of the following two ways: the dead fragment was either grafted into a host gastrula, or it was explanted into a suitable salt solution and brought into contact with a flap of living gastrula ectoderm. The two different methods yielded different results, the

difference apparently being highly significant. Following the former procedure, inductions were obtained in the host germ in conjunction with the graft, showing a high degree of complexity and some organization. They contained in excess brain parts, nose placodes, eyes, lenses, ears, ganglia, balancers, and mesenchyme. As a result of the second method, merely histological differentiation of the explanted ectoderm exposed to the dead fragment into nervous tissue was observed. That is, the cells assumed the shape of medullary cells and were arranged into irregular plates, folds or tubes of unmistakably nervous character, but no further organization into definable organs took place, except in one case where a structure with the appearance of a nose placode was observed.

Spemann, Fischer and Wehmeier (1933) tested the persistence of inductive capacities in fragments of germs which had been killed by chemical means. Treatment with acetone (4 days), acetic acid (1½ hours) and ether (4½ hours) did not destroy the inductive principle. Holtfreter, later (1934a) showed that even material that had been kept in alcohol for 6 months, then carried through xylene, paraffin (60°C.), and back into alcohol, had not lost its inductive capacity, nor was this capacity abolished by several hours' stay in 20 per cent hydrochloric acid or several days' stay in ether.

One of the most surprising results of these experiments was the observation that some tissues which had been known to be completely ineffective while alive would give induction effects once they were killed (Holtfreter, 1933c; Spemann, Fischer and Wehmeier, 1933), for instance, any part of the ectoderm or entoderm of the blastula. Soon it was disclosed that not only embryonic tissues could be used, but that many adult tissues were equally effective, and finally an almost arbitrary number and variety of agents were revealed to possess more or less intense inductive powers, some both in the living and in the dead condition, others only after having been killed. Holtfreter (1934b), Fischer and Wehmeier (1933b), Hatt (1934), Umansky (1932), Woerdeman (1933c) have been successful in obtaining inductions in urodele germs with fragments taken from one or the other of the following organisms and organs:

Worms (Enchytraeus): body fragments.
Snails (Planorbis, Limnaea): foot muscles, hepatopancreas.
Daphnia: coagulated body extract.
Lepidoptera (Deilephila): hemolymph and ganglia of pupa.
Dragon-fly (Libellula larv.): fat body, ganglia.

Fishes (Gasterosteus): heart, liver, ovarian eggs, muscle, spleen.
Amphibia (Triton, Salamandra, Rana): liver, heart, ovarian eggs, muscle, cartilage, brain, retina, regeneration blastema.
Reptiles (Lacerta): liver, kidney, testis.
Birds: liver, kidney, testis, thyroid, fat body, brain, retina; coagulated extract of chick embryos; fragments of primitive streak.
Mammals (mouse): heart, liver, kidney, adrenals, brain, lens; calf's liver.
Man: liver, brain, kidney, thyroid, tongue, sarcoma, carcinoma.

No inductions were obtained with starch (prepared from wheat, potato, banana), agar, chick albumen, lard, wax, charcoal, gelatine, cholesterin, yeast, coagulated frog's blood.

It becomes quite evident from these results that the activating principle in the phenomena of "induction" (Waddington's "evocator") must be a very general property of animal tissues, alive or dead. At the same time, the conclusion seems to be cogent that the organizing principle, in these experiments, was provided not by the "inductor" but by the reacting system, to put it in hypothetical form: by activated fields of the host. The strongest argument for this view can be drawn from the fact that the quality of the induced productions corresponds with a certain approximation to their location in the host germ (Holtfreter, 1934a, b). The appearance of induced eyes and ears, for instance, was restricted to the anterior parts of the host, while tails or kidneys were never found in the head region, although frequently occurring in posterior parts. A statistical survey of the experiments specifically substantiates this: A particular organ (induced) may, in a given case, appear at considerable distance from the corresponding normal organ, the latter serving to indicate the centre of its field; but, if all the cases are compiled, it is found that the frequency with which an organ appears in various levels of different hosts reaches a distinct peak at approximately the level of what we know to be the field for that particular type of organ (Holtfreter, 1934b).

Whereas a connection is thus indicated to exist between the character of an induced formation and its site in the host, there was, on the other hand, no indication whatsoever that the origin of the tissue used as inductor might have anything to do with the specific characters of the formation it had initiated. This consistent lack of specificity in the effects of dead inductors is a general observation, apparently one of the very few features which the bewildering number of inductors, examined so far, have consistently in common.

Unspecific though the activation through dead inductors is, it cer-

tainly is not of all-or-none character. One finds a full range of sizes and degrees of perfection in the various inductions, from the mere appearance of inconspicuous epidermal thickenings to large bulging humps, containing complex masses of supernumerary organs. The assumption that these differences may be due to differences in the intensity of the activating agents rather than to accidental circumstances, seems to be justified, for it has been found that certain agents will, with a certain regularity, evoke extensive inductions, while another kind of agents, under otherwise comparable conditions, will induce only very poor formations (Holtfreter, 1934b). It was also observed that the kind of agents by which inductions were obtained most easily or most frequently also yielded the largest inductions. These observations indubitably prove that the quantitative character of inductions really expresses the strength of the activating influences of the inductor. On the whole, there is a certain parallelism between the size of the induced formations and their complexity and morphological perfection (Holtfreter). If the complexity reached simply depended upon the amount of material available initially, a direct proportion between the intensity of the inducing agent and the amount of material activated by it is all one has to assume, in order to explain the facts. Applying this scale for estimating the intensity of inductive agents, it was found that the effectiveness of an inductor is considerably lowered by heating it over 100°C. and by prolonged extraction with various chemicals. But not until the heating is carried beyond 140°C. is the inductive capacity completely lost (Holtfreter, 1934a).

Once it had been proved that dead materials could exert inductive effects, it seemed a promising task to attempt to isolate the active principle involved and to determine its presumably chemical nature. Attempts in this direction were made simultaneously by Spemann, Fischer and Wehmeier (1933) and by Waddington, Needham and Needham (1933).

The active principle can be dissolved out of the tissues containing it; this is clearly demonstrated by the inductive capacities found in centrifuged watery extracts from eggs (Triton), embryos (chick, Holtfreter), frog muscles, mammalian liver, pancreas, and thymus (Wehmeier, 1934). The fact, however, that boiling (Holtfreter) or keeping the tissue (after acetone fixation, 7 hours) in water for seven days does not abolish its inductive power indicates that the solubility in water of the principle is not complete. Similar results were obtained with other solvents.

From the fact that a tissue, after treatment with alcohol, acetone,

acetic acid, or alcohol-ether, did not lose its inductive capacities, Spemann, Fischer and Wehmeier (1933) had concluded that the active substance was not soluble in any of the mentioned solvents. Needham, Waddington and Needham (1934), however, question the conclusiveness of this argument on the ground that the active principle, even if it were soluble, might not have been completely extracted from the residue which still gave inductive effects. This criticism proved to be justified as Fischer and Wehmeier (1933b) found that not only the residue, but also the acetone extract had inductive capacities.

Needham, Waddington and Needham (1934) prepared extracts from crushed neurulae or older stages. By strong centrifugation the pulp could be separated into three distinct layers: a fatty layer, an opaque aqueous layer, and a coarse residue. Coagulated parts of the cell-free middle layer, which is an aqueous protein fraction, when implanted into a host, induced the formation of medullary tubes. In further experiments, the eggs were extracted with ether, and the extracts, in order to make them suitable for implantation, were solidified by adding substances such as tripalmitine or trioleine. Again the induction of neural tubes was obtained with these grafts. Control experiments, performed with various substances used as carriers for the active extracts, i.e., with agar, celloidin, tripalmitine, trioleine, etc., in various combinations (compare also Wehmeier), yielded no inductions, so that the positive effects in the former series can be rightly ascribed to the substances extracted from the embryos. Ether extracts from adult viscera were also effective. The authors concluded that the active principle was an ether-soluble substance. In a later paper, Waddington, Needham, Nowinski, Needham and Lemberg (1934) reported that the active substance was contained in the unsaponifiable fraction of the saponified ether extract, and that after precipitating that fraction with digitonine the active principle was found in the precipitate. Slightly positive effects were also obtained with two synthetic compounds of the phenanthrene group. Barth (1934) obtained inductions of neural formations in Amblystoma embryos by cephalin extracted from mammalian brain.

While the admitted tendency of Needham and his co-workers has been to isolate one particular substance as the inductive principle, the experiments of Fischer and Wehmeier (1933b) made it appear as if such an attempt were futile, inasmuch as possibly a number of different agents might yield the same effects. Fischer and Wehmeier tested the efficiency of glycogen which they had suspected of possessing inductive capacities. Some of the examined glycogen preparations gave

positive effects (1933a). But, as Needham, Waddington and Needham (1934) have pointed out, these effects might have been due to impurities contained in the preparations rather than to the glycogen itself, which seems to explain also the frequent failures observed by Fischer and Wehmeier (1933b) when they were working with better purified preparations. Woerdeman (1933c) and Holtfreter (1934b) also failed to obtain positive results with glycogen. Fischer and Wehmeier's idea had been that glycolysis might possibly be the activating principle, with glycogen as its source. The negative results obtained with glycogen do not necessarily invalidate this hypothesis, since the presence of a piece of agar soaked with glycogen does not yet prove that glycolysis of the required sort really took place. With due restraint, pending further experimentation, Fischer and Wehmeier seem to feel that the possibility of a connection between content in glycogen and inductive capacity, probably mediated through increased glycolysis, cannot yet be definitely excluded.

Such a connection had already been suggested previously by Woerdeman (1933a, b) and by Raven (1933). Woerdeman found that, while in the animal hemisphere of the blastula the cells contain a high amount of glycogen, fairly uniformly distributed over the whole area, the glycogen content in the chorda-mesoderm cells at gastrulation is considerably lower, the loss occurring rather abruptly while the cells are being invaginated around the lips of the blastopore. That this obvious connection between invagination and loss of glycogen is not mere coincidence was demonstrated by Raven (1933): Even in a transplanted fragment of the blastoporal region, performing its invagination in an abnormal place, the cells were found to lose their glycogen as soon as they became invaginated. Woerdeman contends that the loss of glycogen in the invaginating "organizer" may have something to do with its inductive capacity, and the most probable guess is that glycolysis may be the connecting link (1933b). This view seems to find support in the fact that fragments of malignant tumors (Walker rat carcinoma 256, Peyton-Rous chicken sarcoma), the high glycolytic activity of which is well known, gave positive induction effects (Woerdeman, 1933c). Furthermore, Fischer and Wehmeier (1933b), aware of the rôle of adenylic acid in muscle glycolysis, tested this substance and obtained clear induction effects. While these experiments seem to corroborate the glycolysis hypothesis, Wehmeier (1934) reports that the inductive capacity of the frog's retina, another tissue of high glycolytic activity, is extremely weak.

Since the foundations of the hypothesis that glycolysis may be the

potent tissue activator are still controversial and fragmentary, it would be premature to accept it definitively. But if we compare the implications and consequences of this idea with what the mere detection of a single substance would have to offer, we must admit that there is more appeal to the former conception in that it suggests, at least, in which way the substances involved might give rise to activities and might cause modifications of the development of the parts in their vicinity. The stimulating effect of locally increased metabolic activity has been emphasized so strongly by Child (1929) that his theory at once comes to our mind in this connection. In fact, Huxley (1930) has called attention to the similarity between the effects of "dominant regions," in the sense of Child, and of "organizers" (compare also Huxley and de Beer, 1934).

It seems, however, that the new findings, before being assimilated by any current theory, should first be thoroughly scrutinized as to what their real meaning is. A certain confusion seems imminent, but can be avoided if it is clearly understood to what extent all this newly gathered information on "inductions" may have a bearing on the problem of organization. The indiscriminate use of the term "induction," which has become more or less common practice, is decidely misleading. Surveying the experiments in which "inductions" were obtained, at least four different types must be distinguished:

1. Inductions by live "organizers" in host germs.
2. Inductions by dead "organizers" or other tissues in host germs.
3. Inductions by extracts from dead "organizers" or other tissues in host germs.
4. Inductions by dead "organizers" in isolated explants.

In the first case, organized inductions are observed the quality of which, in characteristic cases, is determined by specific effects of the accessory "organizer" (see p. 651).

In the second and third cases, organized inductions are obtained the quality of which is not determined by the inductor (see p. 660).

In the fourth case, inductions are obtained which cannot be considered as organized.

These statements require some explanation. Concerning the first point, a full discussion was given above. The second and third case apparently can be merged into one, for the reason that, as far as the quality of the induction is concerned, no difference was noted between the effects of dead "organizers" and their extracts, although, speaking in quantitative terms, the extracts generally gave weaker effects. Qual-

itatively, the results obtained with all sorts of dead tissues or their extracts are identical, consisting of the initiation of formative activity in the host tissues exposed to them. In this group should be included also those cases where living adult tissue (e.g., liver, or heart) was effective, the effects, however, being as unspecific as those obtained with the same tissue when dead. In this group apparently belong, furthermore, Balinsky's cases, although celloidin, which in his experiments had provoked the formation of extra limbs, proved ineffective when acting on ectoderm of younger stages.

What this whole group of most diverse agents has in common, is the absence of specific relations between the inductor and the induced formation. So far, nothing has been found that would indicate that particular dead tissues, or their extracts, would give rise to particular types of organs or structures. Whatever organization was acquired by the induced formations, could be traced, at least grossly, to organizing effects of the host's fields, never to determinative influences of the inductors. This view seems to be shared by Needham, Waddington and Needham (1934), and, more conditionally, by Holtfreter (1934a, b). On its rejection or acceptance largely hinges the decision as to whether or not a real bearing on the problem of embryonic organization can be ascribed to the induction effects. The experimental evidence presses toward acceptance. Even cases that do not seem to fit, lend themselves to a plausible interpretation as long as we keep in mind that all our knowledge about the fields, their extension, activity and ontogenetic history, is still utterly fragmentary and vague. For instance, the fact that various induced organs are occasionally found at some, sometimes even at considerable, distance from what one would suppose to be the center of the corresponding host field, merely indicates the wide extension and overlapping of fields, rather than constituting a serious difficulty for our contention.

There is another observation which seems to favor the view that it is the host fields and not the inductors which are responsible for the organization of the extra-formations in this group of inductions: While, in the majority of cases, the inductions were fairly complex structures, containing neural organs, eyes with lenses, ears, noses, balancers, etc., a few cases were observed in which only individual balancers or individual lenses had been induced, with no other organs associated with them (Holtfreter, 1934b). In order to account for this occurrence, we must remember that the individual eye fields, nose fields, ear fields, and balancer fields, are products of emancipation from an original

anterior medullary field (see p. 655). It is conceivable, then, that while in earlier stages the activated material may be exposed to an undivided medullary field which will develop its dependent fields (eyes, ears, etc.) only subsequently, materials activated at a later period will be exposed no longer to the total medullary field but to some of its derivatives, partial fields, such as lens and balancers. Mangold has already been aware of the fact that, to use our terms, different fields are active in the germ at different stages, and F. R. Lillie's (1929) principle of segregation in time would seem to have similar implications. It follows that, the older the host is at the time when the inductions arise, the less complex and the more narrowly circumscribed will their character be. As a matter of fact, Holtfreter specifically states that in the two cases where isolated lenses were induced, the host had been in a comparatively late stage at the time of operation (1934b, p. 320). But even if all the hosts were operated in the same stage, the results may vary, since what counts is not the time when the inductor is put into the host but the time when the activation takes place. Holtfreter (1933c) has found that, in order to obtain induction effects by dead "organizers" in vitro, the reacting tissue must have been exposed to the inductor for at least one day. This observation, without permitting a direct estimate of the efficiency of inducing agents operating in an organism, at any rate suggests that they work rather slowly. It is conceivable, though not yet demonstrated, that weaker factors must operate for proportionately longer time than stronger factors in order to yield effects. Consequently, the weaker the activating agent is, the further the emancipation and subdivision of the host fields will have progressed by the time the agent reaches the effective threshold value. Holtfreter has given due consideration to this point (1934b, p. 367). Inductive agents of different intensities, becoming effective at different times, may therefore indirectly yield products of different complexity.

The experimental evidence, though still fragmentary, is overwhelmingly in favor of the assumption that the connection between dead inductors and their products is entirely unspecific. It is not quite comprehensible, therefore, on what grounds Holtfreter postulates that there must be something more specific to the inductive effects; he ventures to assume that the induction of neural organs and that of mesodermal organs may be due to two different substances (1934b, p. 363). This view which has not been substantiated by any decisive fact seems rather devious. It apparently arose from the preconception that the activating agent, set free by dead inductors, is at the same time an

organizing agent, or in other words, that organization is merely a series of inductions of the sort exhibited by dead inductors. This, indeed, is the salient point of the whole discussion. To be sure, the fact that the effects of living "organizers" and dead inductors are essentially different, in that the former are very specific while the latter apparently are not, has been acknowledged by every one who has worked on the matter. But in the interpretation of this fact, the authors differ significantly among each other. Holtfreter, well aware of the fact that living inductors can exert a variety of specific activities, tries to account for it by postulating the existence of a corresponding variety of inductive substances (1934a, p. 296), which seems to be an utterly deceptive inference. All one can rightly say is that dead inductors merely activate, while live embryonic fragments do both, activate and organize; to conclude, however, that in the latter case the activating and organizing factors must be both of the same order and nature, and to generalize from one to the other does not seem to be a legitimate procedure. Waddington (1934), noting the significant difference between the effects of live and dead "organizers" in the chick embryo, is more cautious in his conclusions and clearly distinguishes between the "evocating" activity of the inductor and the "individuating" activity of the host field, all the organizing, i.e., pattern-determining, effects going to the credit of the latter.

It appears that this is the only stand one can safely take in view of such facts as have come to our knowledge so far. As one can readily see, this implies an admission slightly disappointing: namely, that in regard to the problem of organization no essential progress has been made by all these fascinating results obtained with dead inductors and extracts. The inductive principle seems to be as loosely connected with its product as the kindling spark is with the pattern of fire-works, or to choose a more pertinent example, as the activating acid in artificial parthenogenesis is with the pattern of development it initiates. Like the sperm in fertilization, which performs a double rôle, as activator of the egg and as transmitter of the paternal gene complex, whereby only the activating part can be reproduced by dead agents, so a living "organizer" seems to carry a double rôle, as activator and as organizator, again only the activating component being reproducible by dead inductive agents. And if the activating principle is demonstrated to be carried by a chemical substance, as all these recent experiments seem to prove, this cannot in any sense be taken to mean that organization also is brought about simply by a number of diffusing chemical

substances. As a matter of fact, it is hardly conceivable how it could be so, if the equipotency of the genic endowment of the individual cells of the system is taken for granted. It is of no use to point to the localized effects of hormones as a possible analogy, because it is obvious that diffusing hormones could not have any localized effects, unless they were acting on a system in which differential or specific dispositions to respond were pre-localized. It all leads to the same end: The problem of organization is left as obscure as ever; if anything, it has possibly been shifted a little. We may state it in the form of the field problem, but this merely is putting it into more convenient terms.

Our considerations finally carry us back to one of our introductory statements, namely, that determination includes very heterogeneous processes. The main result of the recent work on inductors is that it adds to our list still another group of factors taking part in the elaboration of the embryo: the unspecific activating factors.

In normal amphibian development, their center seems to coincide with the so-called "organizer" which, in a sense, is unfortunate because it precludes a distinct separation between the overlapping activating and organizing factors. It appears that the egg of some insects is a more suitable object for the study of these questions. Seidel (1929, 1932) demonstrated that the egg of some insects possesses on its posterior end a center from which an influence, apparently a substance, spreads anteriorly during the early cleavage stages, setting free for development those parts of the egg which it has passed. Although being indispensable for the activation of the various levels of the egg, this influence has nothing specific to do with the quality and localization of the ensuing differentiations (1931). More recently Seidel (1934) has demonstrated that, in addition to the activating center, there exists another center in the egg with quite different functions, situated on the ventral side at a considerable distance from the first center and apparently intimately concerned with the pattern of embryonic organization. The organizing and the activating centers, which in the amphibia seem to be combined in the same area, are, therefore, spatially well separated in the insects.

Although we have confined our discussion to the amphibia, it may at least be mentioned that birds seem to fall in line. Inductions of neural organs both by live and dead inductors have here been described (Waddington, 1933a, b, c; 1934, Waddington and Schmidt, 1933), suggesting the probability that there exists a similar duality of activating and organizing factors in birds as there is in amphibia. The area of

Hensen's node has been compared to the dorsal region of the blastopore of the amphibia (Wetzel, 1929). The existence of local self-demarcating organizing fields with no rigid boundaries, most of them with their centers in the vicinity of Hensen's node, has been demonstrated by the method of chorio-allantoic grafting (Hunt, 1932). Whether the activating principle has its center also in this region is not known, but it has been noted that fragments taken from the vicinity of the node when grafted to the chorio-allantoic membrane develop better than do fragments taken at some distance from the node (Hunt, 1932).

If the work with dead "organizers" or their extracts did not yield tangible information concerning the organizing principles, considerable progress certainly has been made in the analysis of the activating principle. The activating principle, apparently a substance, can be extracted, although only slowly, by chemical solvents and is not destroyed by moderate heat (145°C.), its thermo-resistance considerably exceeding that of enzymes. One remarkable fact about it is that it seems to work only in immediate contact with the tissues and does not act at a distance. The presence of a thin membrane between the inductor and the responsive tissues precludes induction effects altogether (Holtfreter, 1933). Its analysis being under way along very promising lines, both biological and biochemical, we can be confident that more comprehensive information will be forthcoming soon.

With regard to the organizing principle, we cannot be equally hopeful. If, as now seems to be a well established fact, the basic pattern of the medullary system is laid down through the specific intervention of the entomesodermal substratum, this certainly proves that organization may be spread from one part of the germ to another part by "induction" in the wider sense of the word; but it certainly does not prove that organization must necessarily occur in this way. There still remains the other possibility, namely, that within a more generalized field an emancipation of partial fields takes place without outside interference. It is quite possible that the determination of the early nervous system represents an exceptional rather than a typical case. Therefore, if encouraged by this best known case many workers are now searching for *the* inductors of the various other organs, such an enterprise can be accepted as a sound working hypothesis, but should not be considered as a postulate.

The emancipation of the partial fields seems to correspond approximately to what F. Lillie (1929) calls segregation. We know little about it, but to keep our eyes shut to its existence will not in any way add to

our information. Apparently, the fact stressed by Mangold that at different times in the course of development different organizing sets—fields in our terminology—are found to reside in the egg is a clear indication for the segregation (Lillie, 1929) or emancipation (autonomization, P. Weiss, 1926b) or evolution (Gurwitsch, 1930) of fields in time. The most fruitful experimental approach would, of course, be to study the real conditions, underlying the formation and differentiation of each organ. It must be admitted, however, that except for a few scattered attempts little effort has been made in this direction. A most promising method, quite inadequately evaluated so far, seems to be offered by tissue culture, provided it is critically and analytically handled; it will help to elucidate the possible means by which organizing factors can affect the cells and tissues, reveal the tools of organization, so to speak; one of these, namely, ultra-structural organization of the colloidal ground substances of the embryo has recently been pointed out (P. Weiss, 1932, 1934b). Well developed mechanics of tissue and organ formation are very urgently needed, in order that eventually we may be enabled to abandon the use of symbols in favor of actual knowledge.

The lack of such knowledge is most seriously felt when we attempt finally to interpret the fourth kind of "inductor" actions reported above, that is, those obtained in isolated fragments of explanted ectoderm under the action of dead "organizers" (Holtfreter, 1933, 1934b). We know that ectoderm cells, such as those used in these experiments, can differentiate in various directions if subjected to the proper conditions. In a neutral medium, e.g., in a salt solution, they merely proliferate and form epidermis (Holtfreter, 1931). In an organic, although unspecific, surrounding, such as the orbit of the eye, they may differentiate into neural or mesodermal parts (Kusche, 1929; Bautzmann, 1929a). If brought into contact with a dead inductor, they most commonly differentiate into neural tissue, tubes, and vesicles of neural character, but without organization into recognizable parts. Under one condition, however, namely, when embryonic chick extract was used as inductor, development of muscle and notochord was also observed (Holtfreter, 1934b), as in the experiments with interplantation into the orbit of the eye. A close check of the experimental conditions would probably offer some clue as to why mesodermal structures arose only in this one series and not in the others, but this check is still wanting.

In these mesodermal as well as in the neural inductions, the cytolog-

ical differentiations into muscle fibres, chorda cells, neural cells, are of fairly normal appearance. As in the interplantation experiments, however, the various types of cells are not scattered, but are grouped together with their kind so that elementary tissue units are formed. This raises a very fundamental question: Is the arrangement of cells in a particular tissue solely determined by the properties of the constituent cells, or must we assume that additional factors come in from the surroundings functioning as some sort of a mould? Our ignorance of this point is almost complete, and it is advisable to put the question for each tissue separately.

Concerning the neural tissue, a primary change in the shape of the cells, for instance elongation, would naturally have an effect on the form of the whole plate. Secretory activity of the cells on one of their free surfaces would result in the formation of a cavity in the center of the cellular mass so that structures with resemblance to a neural tube or brain vesicle may arise; there is evidence that such secretory activity is a real occurrence in embryonic nervous tissues (P. Weiss, 1934a). With regard to the chorda cells, it is possible that, once their vacuolization has taken place as a mere cytological differentiation, the turgor alone will cause the cylindrical shape of the whole unit, the individual cells sticking together by virtue of surface tensions. Growth tensions, resulting from the elongation of the notochordal unit, may orient the muscle fibers along the lines of tension, and so forth. This, to be sure, is mere conjecture that will have to be substantiated before being accepted. But all we intend to show is that, taking into account the functional changes associated with cytological differentiations, groups of cells can assume a certain tissue character without outside interference. What still distinguishes these elementary tissues from the differentiations within the organism, is that, when occurring outside of an organism, in the absence of typical fields, they occur in an atypical, disorderly way. The typical pattern of conditions being absent, a haphazard pattern of differentiations is developed.

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PHYSIOLOGICAL REVIEWS

Contents of Volume 13, 1933

K. S. LASHLEY: Integrative Functions of the Cerebral Cortex

H. E. ROAF: Colour vision

L. H. Weed: Positional Adjustments of the Pressure of the Cerebrospinal Fluid

A. H STEINHAUS: Chronic Effects of Exercise

I. DE BURGH DALY: Reactions of the Pulmonary and Bronchial Blood Vessels

G. F. MARRIAN: Recent Advances in the Chemistry and Biological Assay of Oestrin

W. A. JACOBS: The Chemistry of the Cardiac Glucosides

A. CLAUDE AND J. B. MURPHY: Transmissible Tumors of the Fowl

F. J. MCCLURE: A Review of Fluorine and Its Physiological Effects

H. E. JORDAN: The Structural Changes in Striped Muscle during Contraction

J. G. DUSSER DE BARENNE: The Mode and Site of Action of Strychnine in the Nervous System

E. F. ADOLPH: The Metabolism and Distribution of Water in Body and Tissues

C. E. BRUNTON: The Acid Output of the Kidney and the So-called Alkaline Tide

E. G. WEVER: The Physiology of Hearing: The Nature of Response in the Cochlea

T. DEIGHTON: Physical Factors in Body Temperature Maintenance and Heat Elimination

T. B. HANSON: Radiation-Genetics

C. M. GRUBER: The Autonomic Innervation of the Genito-Urinary System

Contents of Volume 14, 1934

E. K. MARSHALL: The Aglomerular Kidney

L. B. FLEXNER: The Chemistry and Nature of the Cerebrospinal Fluid

H. JENSEN AND E. A. EVANS, JR.: The Chemistry of Insulin

R. G. SINCLAIR: Physiology of the Phospholipids

S. W. CLAUSEN: Diet and Infection

W. S. DUKE-ELDER: Application of Physiological Chemistry in the Study of Intraocular Pressure

J. M. PETERSON: Metabolism of the Eviscerate Preparation

E. M. LANDIS: Capillary Pressure and Capillary Permeability

J. C. HINSEY: The Innervation of Skeletal Muscle

G. A. ALLES: The Physiological Significance of Choline Derivatives

R. C. GARRY: The Movements of the Large Intestine

A. C. IVY: Physiology of the Gall Bladder

S. MUDD, M. MCCUTCHEON AND B. LUCKE: Phagocytosis

Contents of Volume 15, 1935

O. R. MCCOY: Physiology of Animal Parasites

C. L. A. SCHMIDT AND D. M. GREENBERG: Transport of Alkaline Earth Elements in the Animal Body

C. M. WILHELMJ: Specific Dynamic Action

W. F. VON OETTINGEN: Manganese, Its Distribution, Pharmacology and Health Hazards

W. H. HOWELL: Present Status of the Problem of Blood Coagulation

W. E. GAREY AND W. R. BRYAN: Variations in White Blood Cell Counts

C. A. ELVEHJEM: Iron and Copper Metabolism

C. E. BILLS: Physiology of Sterols and the Influence of Light on Sterols

W. M. HOSKINS AND R. CRAIG: Recent Developments in Insect Physiology

M. N. RICHTER AND E. C. MCDOWELL: Experiments with Mammalian Leukemia

P. WEISS: Organizers in Embryological Development

F. J. W. ROUGHTON: Recent Work on CO_2 Transport

R. J. S. MCDOWALL: The Nervous Control of the Blood Vessels

PHYSIOLOGICAL REVIEWS

Tentative Contents of Volume 16, 1936

J. Barcroft: Fetal Circulation and Respiration
W. J. V. Osterhout: Electrical Studies on Large Plant Cells
W. O. Fenn: Electrolytes in Muscle
A. N. Drury: Physiological Activity of Nucleic Acid Derivatives
J. R. Murlin: Some New Factors of Energy Metabolism
G. W. Bartelmez: Menstruation
R. Höber: Membrane Permeability to Solutes in Its Relations to Physiology
D. W. Bronk: Unitary Analysis of Reflex Activity
P. Bard: Functions of the Hypothalamus
L. B. Arey: Mechanism of Wound Healing
A. Forbes and H. Davis: Chronaxie
H. S. Liddell: Recent Contributions to the Physiology of the Conditioned Reflex
A. P. Krueger: The Nature of Bacteriophage and Its Mode of Action
C. C. King: Ascorbutic Acid and Vitamin C
H. J. Deuel: Metabolism of Fructose and Galactose
W. O. Nelson: Lactation
W. H. Chambers: Undernutrition and Carbohydrate Metabolism
D. B. Dill: Economy of Muscular Activity
J. F. Fulton and M. A. Kennard: Representation of Auto nomic Functions in the Cerebral Hemispheres
F. Gowland Hopkins: Glutathione
William E. Anderson and H. H. Williams: Rôle of Fat in the Diet
P. B. Hadley: Bacterial Dissociation and Mutation
C. P. Richter: The Psycho-Galvanic Reflex
E. S. G. Barron: Cellular Respiration
R. K. Burns: Experimental Transformations of Sex in Vertebrates
J. J. Morton and W. J. M. Scott: Sympathetic Vasoconstriction in Normal and Pathological Arteries of the Extremities
H. G. Wolff: The Cerebral Circulation
H. M. Carleton: Phagocytosis of Foreign Material in the Lung
E. W. H. Cruickshank: Metabolism of Cardiac Muscle
H. R. Ing: Curariform Action of Onium Salts
F. S. Hisaw and H. L. Fevold: Sex Hormones of Anterior Pituitary
M. C. Bourne: Metabolic Factors in the Production of Cataract
Carl L. Voegtlin: Biochemistry of Malignant Tissues
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